Please Direct All Correspondence to Customer Number **20995**

APPEAL BRIEF

Applicant	:	Kameron W. Maxwell et al.
App. No	:	10/675,225
Filed	:	September 29, 2003
For	:	NITROXIDE RADIOPROTECTOR FORMULATIONS AND METHODS OF USE
Examiner	:	James William Rogers
Art Unit	:	1618

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the Notice of Appeal filed March 15, 2007, Applicant submits this Appeal Brief.

TABLE OF CONTENTS

I. REAL PARTY IN INTEREST

Pursuant to 37 C.F.R. § 1.192, Appellants hereby notify the Board of Patent Appeals and Interferences that the real party in interest is the assignee of this application: Mitos Pharmaceuticals, Inc., 3 San Joaquin Plaza, Suite 200, Newport Beach, CA, 22660.

II. RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

III. STATUS OF CLAIMS

The above-identified application was filed with 25 claims. Claims 1-25 were rejected by the Examiner in an Office Action mailed April 11, 2006. Subsequently, Claims 11, 13, 16, 24, and 25 were amended and Claim 18 was canceled. Claims 1-17 and 19-25 were finally rejected in an Office Action mailed September 15, 2006. Subsequent to that Office Action, Claim 24 was amended. The amendment to Claim 24 was not entered for purposes of appeal by the Examiner in an Advisory Action mailed February 16, 2007, in which the Examiner indicated that Claims 1-17 and 19-25 remained rejected. Accordingly, Claims 1-17 and 19-25 are the subject of this appeal. The claims are attached hereto as Section VIII.

IV. STATUS OF AMENDMENTS

In the Advisory Action mailed February 16, 2007, the Examiner indicated that the amendments filed subsequent to the final rejection of the claims would not be entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter relates to radioprotective nitroxide compositions used during radiation treatments. Prior art compositions caused topical burning during radiation treatment; use of the claimed compositions allows the amelioration or avoidance of topical burning resulting from the bolus effect. The composition may be in the form of a low-residue gel or low-residue thickened liquid. The nitroxide-containing composition may be applied to skin, and the solvent therein allowed to evaporate before applying radiation. *See, e.g.*, specification at ¶¶ [0060] – [0065].

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The Examiner has rejected Claims 1-2, 6-10, 12-18, and 20-25 under 35 U.S.C. § 102(b) as being unpatentable over Mitchell et al., United States Patent No. 5,462,946, and has rejected Claims 1-25 under 35 U.S.C. § 103(a) as being unpatentable over Mitchell et al., in view of Golz-Berner et al., International Patent Application Publication No. WO 99/66881 (using United States Patent No. 6,426,080 as an equivalent).

VII. ARGUMENT

The Examiner has maintained his rejection of Claims 1-2, 6-10, 12-18, and 20-25 under 35 U.S.C. § 102(b) as being unpatentable over Mitchell '946, and has maintained his rejection of Claims 1-25 under 35 U.S.C. § 103(a) as being unpatentable over Mitchell '946, in view of Golz-Berner '080. The Examiner maintained the rejection based on allegations that:

(A) although Mitchell's disclosed topical formulations are limited to liquids, ointments, lotions, or creams, Mitchell nevertheless discloses both "gel" and "thickened liquid" formulations;

(B) although Appellants' originally-filed specification specifically distinguished Mitchell's topical "ointment, lotion, or cream" formulations as leaving a significant residue on the skin, Mitchell nevertheless discloses a "low residue" formulation; and

(C) one of skill in the art would employ certain solvents disclosed in Golz-Berner to produce low-residue formulations of the active ingredient disclosed in Mitchell, even though Golz-Berner indicates that other ingredients are to be included in those compositions and the presence of those ingredients would leave a significant residue on the skin.

Each of these allegations is addressed below.

A. Claims 1-2, 6-10, 12-18, 20-23, and 25 Are Not Anticipated By Mitchell '946

1. Mitchell Discloses Neither A "Thickened Liquid" or a "Gel" Formulation

In order to establish a *prima facie* case of anticipation, the Examiner is required to demonstrate that all of the limitations of the claims are present in a single prior art reference. *See* M.P.E.P. § 2131. The Examiner has failed to do this. Indeed, the Examiner has essentially

ignored the "thickened liquid" and "gel" limitations that are present in almost all of the pending claims.

a. Applicant's Claims Require A "Thickened Liquid" or a "Gel" Formulation, Which Are Described In Applicant's Specification

Independent composition Claims 1 and 15 require that the pharmaceutical composition be "in the form of a low-residue gel." Independent composition Claim 13 requires that the pharmaceutical composition be "in the form of a low-residue gel or low-residue thickened liquid." Independent method Claims 16 and 25 require that the solution be "in the form of a low-residue gel or low-residue thickened liquid." Thus all of these claims, and their dependent claims, require that the claimed composition, or composition employed in the claimed method, be in the form either of a "gel" or a "thickened liquid." For greater clarity, Applicants further described both of these forms in the specification.

A "gel" is a "semisolid system of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Generally, if left undisturbed for some time, gels may be in a semisolid or gelatinous state." Specification at ¶ [0091]. Gels will also "typically comprise a major amount of a liquid phase and a minor amount of a thickening or gelling agent." Specification at ¶ [0064].

According to Applicants' specification, a thickened liquid may be obtained by adding polymers to a nitroxide-containing solution to achieve a dynamic viscosity of 20-100,000 or more centipoise. Specification at ¶¶ [0099], [0101].

Furthermore, Applicants clearly distinguished both the gel and thickened liquid formulations from other formulations, such as "creams, lotions, shampoos, cream rinses, and ointments," which "are unsuitable for administration shortly before the actual delivery of radiotherapy to the patient. Indeed, these product forms leave residues that can result in topical burning, including severe burns, when radiation is administered." Specification at ¶ [0009].

b. The Examiner Ignored Both The "Gel" and "Thickened Liquid" Limitations and Applicant's Relevant Disclosure

It is axiomatic that, in order for a printed publication to anticipate a claim, that reference must disclose elements corresponding to each limitation of the claim. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 814 F.2d 628, 631 (Fed. Cir. 1987). The Examiner was confronted with claims that clearly were limited to "gel" or "thickened liquid" formulations, and had the benefit of clear guidance as to the meaning of those limitations and how they could be distinguished from other formulations. The Examiner's citation of Mitchell '946 as an anticipating reference is, therefore, only proper if it discloses a pharmaceutical composition that is in the form of a "gel" or a "thickened liquid." It does not. This rejection is unjustified, and under these circumstances is frankly incomprehensible to Applicants.

The Examiner began his substantive prosecution of this case by identifying Mitchell's disclosure of a topical "ointment, lotion, or cream" as "satisfying the claim for a gel or thickened liquid." Office Action mailed April 11, 2006 at 2. Applicants responded by directing the Examiner's attention to the relevant portions of their specification, which indicated that Mitchell's topical formulations were in fact not gels or thickened liquids, and that ointments, lotions, or creams such as those disclosed in Mitchell would cause burning on delivery of radiotherapy, avoidance of which was a primary motivation to create the claimed invention. Response of August 11, 2006 at 7. The Mitchell formulations clearly did not fall within the literal scope of the "gel" or "thickened liquid" limitations. The Mitchell formulations' unsuitableness for the claimed purpose reinforced this conclusion. Applicant therefore requested that the Examiner recognize that Mitchell failed to disclose "gel" or "thickened liquid" formulations, and withdraw the rejection under Section 102. Id. at 9.

The Examiner responded by noting Applicants' assertion that Mitchell did not disclose formulations corresponding to these limitations, but stating that "the relevance of this assertion is unclear." Office Action mailed September 15, 2006 at 2. In so doing, the Examiner implicitly denied the "gel" and "thickened liquid" limitations any patentable weight, which was clearly improper. Evidently, however, the Examiner did recognize that his first rejection was somehow inadequate, because he went on to cite other Mitchell formulations, specifically "an aerosol drop or spray," which he maintained would also "satisfy the limitation of a low residue gel or low

residue thickened liquid." *Id.* at 3. Unlike the ointments, lotions, and creams originally cited by the Examiner, the aerosols, drops, and sprays of Mitchell are not topical formulations designed to protect the skin, but are rather for inhalation, placement in the eyes, or application to plants. Mitchell '946 at col. 2, line 63 – col. 3, line 30. Furthermore, none of these forms meets either the "gel" or "thickened liquid" limitations.

Mitchell '946 does contain a brief reference to a topical "liquid" formulation. *See* Mitchell '946 at col. 5, line 19. However, there is simply no disclosure of a "thickened liquid," and that is what is claimed. Furthermore, Applicant's specification clearly indicates that "liquids" are to be distinguished from "thickened liquids," since it discusses them as separate alternatives. *See* specification at ¶¶ [0062], [0069], [0079], [0098]-[0101]. Mitchell's brief mention of a topical "liquid" formulation is therefore insufficient to meet the "thickened liquid" limitation.

2. <u>Applicant's Claims Also Require A "Low-Residue" Formulation, Which Is Not Disclosed By Mitchell</u>

Claims 1-2, 6-10, 12-18, 20-23, and 25 also require that the formulation be "low-residue." The Examiner appears to have ignored this limitation of the pending claims as well, and has identified no disclosure in Mitchell that corresponds to this limitation. Indeed, Mitchell appears to have been unaware of the problem of topical burning caused by such residues.

As the present specification makes clear, prior art creams, lotions, shampoos, cream rinses, and ointments such as those disclosed in Mitchell leave residues on the skin that can result in severe burning when applied shortly before the administration of radiotherapy. *See* specification at ¶ [0073]. The avoidance of the problem of residue-induced burning by the described and claimed low-residue formulations was recognized by the Applicant. *See* specification at ¶ [0064]. Indeed, "low-residue" is defined in terms of such burning in the specification: "[a]s used herein, 'low-residue' refers to formulations that can be applied to a patient, shortly before undergoing radiotherapy, without leaving a residue capable of enhancing a bolus effect upon delivering radiotherapy to the treated area." Specification at ¶ [0084].

The Examiner has consistently failed to identify any disclosure of a "low-residue" formulation in Mitchell. Nor has he identified any motivation to modify the disclosures of

Mitchell to make a low-residue formulation. Instead, he has simply pointed to the disclosure in Mitchell of topical ointment, cream or lotion formulations (or aerosol drop or spray formulations) as somehow satisfying the "low-residue" limitation. *See, e.g.,* Office Action mailed April 11, 2006 at 2; Office Action mailed September 15, 2006 at 3; Advisory Action at 2. Yet these are the same formulations specifically distinguished in the present specification as not being "low-residue" formulations. *See* specification at ¶ [0009] (specifically describing prior art including Mitchell as "limit[ing] the topical use of Tempol to formulations selected from creams, lotions, shampoos, cream rinses, and ointments" that "leave residues that can result in topical burning, including severe burns, when radiation is administered."). Applicant states that Mitchell's formulations are not low-residue; in effect, the Examiner tells the Applicant that he is wrong. The Examiner has no apparent basis to do so.

Applicant did direct the Examiner's attention to the need for a "low-residue" disclosure during prosecution. In response to the Examiner's final rejection over Mitchell, Applicant provided the Examiner with further evidence of standard definitions of terms such as "ointment" and "cream." Amendment and Response filed January 16, 2007 at 6-7. Applicant noted that the U.S. Food and Drug Administration's Center for Drug Evaluation and Research Data Standards Manual defines an "ointment" as "[a] semisolid dosage form, usually containing <20% water and volatiles and >50% hydrocarbons, waxes, or polyols as the vehicle." Furthermore, it defines a "cream" as "an emulsion, semisolid dosage form, usually containing > 20% water and volatiles and/or < 50% hydrocarbons, waxes, or polyols as the vehicle." As Applicant argued, dosage forms containing such significant amounts of residue-producing substances will not be "low-residue." Neither are "lotions" limited to low-residue forms: they are defined by the FDA simply as "[a]n emulsion, liquid dosage form." Indeed, as Applicant noted, guidelines to patients undergoing radiation therapy generally counsel specifically against the use of lotions on the treated area during therapy.

Accordingly, the Examiner has filed to identify a disclosure in Mitchell '946 that corresponds to the "low-residue" limitation of Claims 1-2, 6-10, 12-18, 20-23, and 25, and the rejection of those claims over Mitchell must fail on this ground as well.

3. The Examiner's Final Anticipation Rationale Does Not Apply To Claims 1-12 and 15

In response to Applicant's January 16, 2007 response, the Examiner issued an Advisory Action. In explaining his rejection over Mitchell, the Examiner made a candid and rather startling admission relevant to his examination of these claims. Specifically, he indicates that he focused on the "thickened liquid" limitation to the exclusion of the "gel" limitation:

> Thickened liquid or gel was interpreted in the broadest reasonable way by the examiner therefore the recitation of "thickened" is not considered to be very limiting. The examiner searched thickened liquid or gel to mean any composition that contained a solvent or a solution in which the solvent/solution was more viscous or thickened after addition of the ingredients, for example to make a cake one would use milk and flour, upon mixing milk with flour the batter is more thickened or viscous than just milk alone, the limitation was interpreted in a similar manner. Since an ointment, cream or lotion is thicker or more viscous than a solvent or solution the limitation is considered met.

Advisory Action of February 16, 2007 at 2 (emphasis added).

The "thickened liquid" limitation is, however, entirely absent from Claims 1-12 and 15. The Examiner's reasoning does not apply to those claims. The Examiner here maintains that any sort of composition containing a thickened solution meets the "gel" limitation, including the ointments, creams, and lotions disclosed in Mitchell. As a matter of standard usage in the art, this is clearly wrong. Each of these terms has a distinct meaning, and ointments, creams, and lotions are not gels. *See* Amendment and Response mailed January 16, 2007 at 6-7. The Examiner's conclusion that an ointment, cream, or lotion satisfies the "gel" limitation is incorrect. Accordingly, based on the Examiner's own searching and examination rationale, Claims 1-12 and 15, at least, should be allowable over Mitchell.

Because the Examiner has failed to identify a disclosure in Mitchell of a topical composition in the form of a "gel" or "thickened liquid," or a "low-residue" composition, he has failed to establish a prima facie case of anticipation of Claims 1-2, 6-10, 12-18, 20-23, and 25 by Mitchell, and Applicant requests that this rejection be reversed.

B. Claim 24 Is Not Anticipated By Mitchell '946

Pending Claim 24 recites the steps of "evaporating solvent; and applying radiotherapy to said patient." In rejecting this claim as anticipated by Mitchell, the Examiner stated that "applying the composition topically to prevent harmful effects of radiotherapy is taught by Mitchell (*see* col. 2, lines 53-58) and evaporating solvent after applying topically is inherent since the solvents listed are volatile (methanol) and would eventually evaporate when applied to a person's skin." Office Action mailed April 11, 2006 at 3.

To anticipate Claim 24, evaporation of the solvent in the formulation must take place before the radiotherapy is applied to the patient. Mitchell does not disclose the timing of the application of the topical formulations with respect to the application of ionizing radiation. Furthermore, as recognized by the Examiner, Mitchell is silent as to the specific solvents to be employed. *See* Office Action mailed April 11, 2006 at 2. It is possible that, if applied well in advance of the application of ionizing radiation, the undisclosed solvent in the "ointment, lotion, or cream" formulation of Mitchell will evaporate before ionizing radiation is applied. The Examiner apparently recognized that he needed to establish that the solvent would always evaporate before the application of radiotherapy, because he later based his inherency rationale on a speculation that the solvent would "evaporate almost immediately." Office Action mailed September 15, 2006 at 3.

However, "[i]nherency . . . may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999); *see also* M.P.E.P. § 2112(IV). Rather, "the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference." *In re Robertson*, 169 F.3d at 745. Despite the Examiner's speculation, the precise timing of the evaporation of an undisclosed solvent from a composition applied to the skin is simply not ascertainable. Particularly where the timing of application is not disclosed, a disclosure of the evaporation of that solvent before the application of later radiotherapy is not "necessarily present."

Because Mitchell does not disclose either the solvent to be employed or the timing of the application of the topical formulation, a disclosure of evaporation of the solvent before the application of ionizing radiation is not "necessarily present" in Mitchell. For this reason, the Mitchell disclosure does not inherently anticipate Claim 24, and Applicant requests that this rejection be reversed.

C. Claims 1-17 and 19-25 Are Not Obvious In View Of Mitchell '946 and Golz-Berner '080

The Examiner has rejected Claims 1-25 under 35 U.S.C. § 103(a) as being unpatentable over Mitchell '946, in view of Golz-Berner et al., PCT Publication No. WO 99/66881. Claim 18 was canceled in the Response filed on August 15, 2006. Because the Examiner has failed to make out a *prima* facie case of obviousness over this combination of references, Claims 1-17 and 19-25 are not obvious in view of this combination of references.

1. The Examiner Has Not Established A *Prima Facie* Case Of Obviousness Of Claims 1-17 and 19-25

a. Neither Mitchell Nor Golz-Berner Discloses a "Low-Residue" Formulation

The topical formulations of the present application are designed to leave little residue on the skin after a short period of time, in order to ameliorate or avoid the problem of burning caused by radiotherapy. One of skill in the art would not produce a "low-residue" formulation even by combining the teachings of Mitchell and Golz-Berner.

As noted above, Mitchell discloses the use of a topical radioprotective formulation in the form of an ointment, cream, lotion, or liquid. As discussed above, none of these formulations satisfy the "low-residue" limitations of the claims.

The Examiner relies on Golz-Berner for "its disclosure of cosmetic active substances to protect the skin and the use of solvents, carriers, and hydrogels." Office Action mailed September 15, 2006 at 5. The Examiner states that "it would have been obvious to modify the solvents and carriers of Golz-Berner with the composition of Mitchell, especially since they are related to the same field of endeavor." Advisory Action of February 16, 2007 at 2. In essence,

the Examiner takes Golz-Berner's disclosure of the use of water and certain glycols in cosmetic preparations to be the equivalent of the disclosure of a low-residue gel. It is not.

Like all references cited in an obviousness rejection, Golz-Berner must be considered in its entirety, including portions that would lead away from the claimed invention. *See W.L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1550-51 (Fed. Cir. 1983); M.P.E.P. §2141.02(VI). When Golz-Berner is considered in its entirety, its disclosure would not lead one of skill in the art to a low-residue gel or low-residue thickened liquid.

Applicant's specification makes clear that the low-residue formulations can be achieved by including only a very minor amount of a gelling agent, which remains behind after evaporation of the solvent together with the active ingredient. *See* specification at ¶ [0064]. Thus "98%, 99% or more of the carrier for the drug can disappear prior to radiotherapy, greatly reducing or eliminating burning due to the bolus effect." *Id.*

In contrast, Golz-Berner is concerned with the preparation of cosmetic preparations, with one stated objective of the invention being "to provide a preparation of active substances that keeps its radical protection potential over a long period of time." Golz-Berner at col. 1, lines 52-54. In its broadest disclosure, Golz-Berner teaches that the preparation achieves an incorporation of the active ingredients in an "association complex" containing not only the hydrogel components identified by the Examiner, but also a significant fraction of phospholipids (up to 30% by weight). *See* Golz-Berner at col. 2, lines 11-12; col. 3, lines 37-42. One of skill in the art, on reviewing the Golz-Berner reference for disclosure of how to prepare a topical radioprotective formulation, would also incorporate these phospholipids into the formulation to form a similar association complex with the nitroxide active ingredient. The Examiner notes that Mitchell does not disclose the use of these phospholipids, *see* Advisory Action at 2, but it is after all the disclosure of Golz-Berner, not Mitchell, that the Examiner must rely on for the disclosure of how to make the low-residue gel or thickened liquid. Furthermore, one of skill in the art would obviously turn to the exemplary formulations, rather than a laundry list of possible ingredients, for guidance in producing the actual composition. The phospholipids, which have a much higher molecular weight than the disclosed solvents, would not readily evaporate, and the

resulting composition would leave a residue that would cause topical burning during radiotherapy.

Furthermore, although the Examiner has treated the examples of Golz-Berner as non-limiting, see Advisory Action at 2, those examples must also be considered for what they would indicate to one of skill in the art. *See W.L. Gore*, 721 F.2d at 1550-51 (Fed. Cir. 1983). Golz-Berner discloses exemplary cosmetic compositions, the majority of which are described as "creams." Golz-Berner '080 at col. 9, line 55 – col. 11, line 28. This term coincides with Mitchell's teaching that a "cream" form should be used for the radioprotective formulation, making it more likely that one of skill would follow the specific teachings disclosed. Each of these exemplary "cream" formulations (as well as formulations described as a "sun gel" and "emulsion-based fluid") contains not only the phospholipid-containing active complex, but also a considerable amount of glycerine. *See id.* Glycerine is highly hygroscopic and will slow the rate of evaporation of the solvents employed in the compositions. Because a significant amount of not only phospholipid but also glycerine is included in each of the exemplary formulations disclosed in Golz-Berner, topical formulations made following the teachings of Golz-Berner would not result in the "low-residue gels" or "low-residue thickened liquids" required by Claims 1, 13, 15, 16, and 25. Neither, given the presence of these ingredients, would the resulting formulations meet the requirements of Claim 24, wherein evaporation of solvent occurs before radiotherapy is applied.

As a result, even if the teachings of Golz-Berner were combined with those of Mitchell, a "low-residue" gel or thickened liquid would not result. Because a disclosure corresponding to this limitation, and the limitation of Claim 24 discussed above, are not found in the cited prior art references, a prima facie case of obviousness has not been established.

2. <u>There is No Apparent Reason to Combine the Reference Teachings</u>

The Supreme Court has recently clarified the law governing obviousness determinations. *See KSR Int'l Co. v. Teleflex, Inc.*, 127 S.Ct. 1727 (2007). The Court stated that as part of the obviousness inquiry, it is necessary to "determine whether there was an apparent reason to combine the known elements in the fashion claimed." *KSR Int'l Co.*, 127 S.Ct. at 1731; *see also*

Ex parte Smith, No. 2007-1925 (Bd. Pat. App. & Interf. June 25, 2007) (precedential opinion adopting *KSR* standard in ex parte prosecution context). In this case, the Examiner has cited no such apparent reason to combine Mitchell with Golz-Berner that did not involve hindsight reconstruction of Appellants' invention.

In initially rejecting Appellants' claims over the combination of Mitchell and Golz-Berner, the Examiner observed that "the motivation to combine the two documents would be the formulation of a pharmaceutical topical gel for use as a radioprotector, the gel composition comprised of TEMPOL, solvent and polymers." Office Action mailed April 11, 2006 at 5. In other words, the Examiner's stated "reason to combine" the two documents was to recreate Appellants' invention, using their own disclosure as a blueprint. It has long been the case that such "hindsight reconstruction" is impermissible, and Appellants are not aware that recent Supreme Court precedent has changed the law in this area. Early in its existence as a court, the Federal Circuit succinctly described hindsight reconstruction and its prohibition: "When prior art references require selective combination by the court to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself." *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1143 (Fed. Cir. 1985). Because the Examiner offered no reason to combine Mitchell and Golz-Berner other than in order to make Appellants' invention in the manner described by Appellants themselves, the combination is improper.

Furthermore, the Examiner characterizes the two references as being "from the same field of endeavor," Advisory Action at 2, but this is surely too facile. As noted above, Golz-Berner is concerned with the preparation of cosmetic preparations that contain a number of extracts from plants and insects, whereas the relevant disclosure of Mitchell is concerned with the preparation of a composition for the specific medical use of protecting against ionizing radiation such as that used in radiotherapy. One of skill in the art would be unlikely to turn to known cosmetic formulations for guidance in producing a very different medicinal formulation that would be used on skin subject to the high levels of radiation encountered in radiotherapy.

Claims 1-17 and 19-25, as presently amended, are not obvious over the cited prior art, and reversal of this rejection is respectfully requested.

CONCLUSION

In view of the arguments presented above, appellants submit that the pending claims are not obvious in view of the cited prior art combination and respectfully request that the rejections under Sections 102(b) and 103(a) be reversed, and that the application be allowed.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: October 15, 2007

C. Philip Poirier
Registration No. 43,006
Attorney of Record
Customer No. 20,995
(949) 760-0404

VIII. CLAIMS APPENDIX

1. (Original) A pharmaceutical composition for use in ameliorating an effect of radiotherapy on skin, mucous membranes, or hair follicles comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

2. (Original) The pharmaceutical composition of Claim 1, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

3. (Original) The pharmaceutical composition of Claim 1, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

4. (Original) The pharmaceutical composition of Claim 3, wherein the solvent is an alcohol selected from the group consisting of methanol, ethanol, propanol, and butanol.

5. (Original) The pharmaceutical composition of Claim 3, wherein the glycol is selected from the group consisting of ethylene glycol and propylene glycol.

6. (Original) The pharmaceutical composition of Claim 1, wherein the effect of radiotherapy is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity, and polynucleic acid damage.

7. (Original) The pharmaceutical composition of Claim 6, wherein the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

8. (Original) The pharmaceutical composition of Claim 6, wherein the mucous membrane condition is selected from oral mucositis and proctitis.

9. (Original) The pharmaceutical composition of Claim 6, wherein the hair follicle condition is alopecia.

10. (Original) The pharmaceutical composition of Claim 1, wherein the effective prophylactic or therapeutic amount of a nitroxide radioprotector is an amount from about 0.01 to about 100 mg/ml of the total composition.

11. (Previously presented)The pharmaceutical composition of Claim 1, further comprising a polymer selected from the group consisting of ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, natural polymers, polystyrene polymers, silicone polymers, and inorganic polymers.

12. (Original) The pharmaceutical composition of Claim 1, having a viscosity such that the nitroxide radioprotector will remain in contact with a treated area for a sufficient period of time to allow absorption of a pharmacologically effective amount into said treated area.

13. (Previously presented) A pharmaceutical composition for use in ameliorating an effect of radiotherapy to skin or mucous membranes, comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel or low-residue thickened liquid that does not leave an amount of residue sufficient to enhance burning to the skin or mucous membranes when radiotherapy is applied.

14. (Original) The pharmaceutical composition of Claim 13, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

15. (Original) A pharmaceutical composition for use in preventing or treating alopecia comprising:

a solvent; and

an effective prophylactic or therapeutic amount of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

16. (Previously presented) A method of treating a patient, comprising topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat harmful side effects caused by radiotherapy, wherein the nitroxide radioprotector is in solution in a solvent, and the solution is in the form of a low-residue gel or a low-residue thickened liquid.

17. (Original) The method of Claim 16 wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

18. (Canceled)

19. (Original) The method of Claim 16, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

20. (Original) The method of Claim 16 where the harmful side effect is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity and polynucleic acid damage.

21. (Original) The method of Claim 20 wherein, the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

22. (Original) The method of Claim 20 wherein, the mucous membrane condition is selected from oral mucositis and proctitis.

23. (Original) The method of Claim 20, wherein the hair follicle condition is alopecia.

24. (Previously presented) A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent;

evaporating solvent; and

applying radiotherapy to said patient.

25. (Previously presented) A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent, has a sufficient viscosity such that it is retained in place on the patient, and the solution is in the form of a low-residue gel or a low-residue thickened liquid; and

applying radiotherapy to said patient.

IX. EVIDENCE APPENDIX

1. Specification as filed;
2. Office Action mailed April 11, 2006;
3. Response to Office Action, filed August 11, 2006;
4. Office Action mailed September 15, 2006;
5. Response to Office Action, filed January 16, 2007;
6. Advisory Action of February 16, 2007;
7. United States Patent No. 5,462,946;
8. United States Patent No. 6,426,080.

X. RELATED PROCEEDINGS APPENDIX

There are no decisions rendered by a court or the Board in any related proceedings identified above.

3883544
061407

NITROXIDE RADIOPROTECTOR FORMULATIONS AND METHODS OF USE

Related Applications

[0001] The present application claims priority to U.S. Provisional Application No. 60/415,089, filed October 1, 2002, and U.S. Provisional Application No. 60/429,887, filed November 26, 2002, both of which are expressly incorporated by reference in their entireties.

Field of the Invention

[0002] The present invention relates generally to the field of preventing or treating the negative side effects which accompany radiotherapy. More particularly, this invention relates to the discovery of new formulations that can be applied to the skin and mucous membranes of patients undergoing radiotherapy and methods of using these formulations.

Background of the Invention

[0003] Radiation therapy is an important tool in the fight against cancer and is used in the treatment of as many as 50% of all cancer patients. Accordingly, more than half a million cancer patients receive radiation therapy each year. While the use of radiation therapy is an effective way to treat many kinds of cancer, there are many complications that may result. Common complications can include negative effects on the patients skin, hair follicles, and mucous membranes .

[0004] Common skin complications of radiotherapy include erythema and folliculitis. These disorders can be very irritating to patients as they both involve pruritus and redness of the skin. These and other skin complications can arise through oxidative and other stress caused by radiation. Other examples of skin conditions caused by radiation include fibrosis, dry desquamation and moist desquamation.

[0005] In addition, hair follicles are quite sensitive to radiotherapy. Accordingly, if hair is in the radiation treatment beam field, it can cease to grow and fall out. Losing one's hair can be a source of embarrassment and loss of self esteem.

[0006] Radiotherapy can also have negative effects on the mucous membranes in the eyes, nose, mouth, vagina, rectal mucosa and the like. For example, oral mucositis, also called stomatitis, results from the local effects of radiation to the oral mucosa. Mucositis is characterized by inflammation of the mucosa of the mouth and ranges from redness to severe ulceration. Symptoms of mucositis vary from pain and discomfort, to an inability to tolerate food or fluids. Even worse, oral mucositis may be so severe as to limit the patient's ability to tolerate further radiotherapy or chemotherapy.

[0007] Patients with damaged oral mucosa and a reduced immunity resulting from radiotherapy are also prone to opportunistic infections in the mouth. Accordingly, mucositis may also further compromise a patient's response to treatment and/or palliative care. It is therefore extremely important that mucositis be prevented whenever possible, or at least treated to reduce its severity and possible complications.

[0008] Another common mucous membrane condition caused by radiotherapy is proctitis. Proctitis is an inflammation of the lining of the rectum (rectal mucosa). The most common symptom is a frequent, or continuous sensation, or urge to have a bowel movement. Other symptoms include constipation, a feeling of rectal fullness, left-sided abdominal pain, passage of mucus through the rectum, rectal bleeding, and anorectal pain.

[0009] Some have previously suggested the use of Tempol, a stable nitroxide radical characterized by the chemical formula 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, as a topical formulation to ameliorate the effects of radiotherapy. (*See e.g.,* Proctor, U.S. Pat. No. 5,352,442, and Mitchell, U.S. Pat. No. 5,462,946, both of which are hereby incorporated by reference in their entireties). These references limit the topical use of Tempol to formulations selected from creams, lotions, shampoos, cream rinses, and ointments. It is now recognized that these kinds of topical formulations are unsuitable for administration shortly before the actual delivery of radiotherapy to the patient. Indeed, these product forms leave residues that can result in topical burning, including severe burns, when radiation is administered. Accordingly, there is a need in the art to provide a topical formulation that can be administered to a patient shortly before the actual delivery of radiotherapy.

Summary of the Invention

[0010] Embodiments of the invention relate to pharmaceutical compositions for use in ameliorating an effect of radiotherapy on skin, mucous membranes, or hair follicles including a solvent and an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, preferably a solvent that is thickened or is in the form of a low-residue gel. Certain preferred embodiments the nitroxide radioprotector are TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl, and TEMPOL, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

[0011] Pharmaceutical compositions can include solvents selected from the group consisting of water, urea, alcohols, and glycols. In embodiments where the solvent is an alcohol, the alcohol may advantageously be selected from the group consisting of methanol, ethanol, propanol, butanol, and the like. In embodiments where the solvent is a glycol, the glycol may advantageously be selected from the group consisting of ethylene glycol, propylene glycol, and the like. In certain embodiments, it is preferred to use water, or other non-irritating liquids, as a solvent for formulations to be administered to the mucous membranes. In additional embodiments, solvents used for mucous membrane formulations are not irritating (e.g., alcohol, urea, and the like).

[0012] In particular embodiments, pharmaceutical compounds described herein can ameliorate conditions caused or enhanced by radiotherapy including skin conditions, mucous membrane conditions, hair follicle conditions, and the like. In specific embodiments the particular skin conditions that the pharmaceutical compositions can treat or prevent include erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, dermatitis, and the like. In some embodiments, pharmaceutical compositions described herein can prevent mucous membrane conditions such as oral mucositis, proctitis, and the like, and are particularly valuable in protecting the rectal mucosa during radiotherapy of tumors in that area, such as prostate tumors. Additionally, in other embodiments the pharmaceutical compositions can treat or prevent hair follicle conditions such as alopecia, and the like.

[0013] In further embodiments, the effective prophylactic or therapeutic amount of the nitroxide radioprotector is an amount from about 0.01 to about 100 mg/ml of the

formulation. Specific examples of particular amounts contemplated include about .02, .03, .04, .05, .10, .15, .20, .25, .30, .35, .40, .45, .50, .55, .60, .65, .70, .75, .80, .85, .90, .95, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 ,35 ,36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more mg/ml. In certain embodiments, the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

[0014] Additional embodiments include pharmaceutical compositions including a polymer selected from the group consisting from ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, natural polymers, polystyrene polymers, silicone polymers, and inorganic polymers.

[0015] Further embodiments include pharmaceutical compositions having a viscosity such that the nitroxide radioprotector will remain in contact with a treated area for a sufficient period of time to allow absorption of a pharmacologically effective amount into said treated area.

[0016] Embodiments of the invention also include pharmaceutical compositions for use in ameliorating an effect of radiotherapy to skin, mucous membranes, or hair follicles including a solvent and an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, preferably wherein the pharmaceutical composition is thickened with a viscosity-enhancing agent, such as carboxymethylcellulose, a gum such as guar gum, an alginate, or other low-residue thickening agent, or is in the form of a low-residue gel. The thickening or gelling agent should be selected so as not to leave a sufficient residue to enhance burning to the skin or mucous membranes when radiotherapy is applied. In certain embodiments, the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

[0017] Additional embodiments include pharmaceutical compositions for use in preventing or treating alopecia including a solvent and an effective prophylactic or therapeutic amount of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

[0018] Other embodiments include methods of treating a patient comprising topically applying a sufficient amount of nitroxide radioprotector to prevent or treat harmful side effects caused by radiotherapy, wherein the nitroxide radioprotector is in solution in a solvent. In preferred embodiments the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl. Other advantageous embodiments include solutions in the form of a low-residue gel or thickened liquid. In certain embodiments, the solvent can be selected from the group consisting of water, urea, alcohols, and glycols. It is preferred that harmful side effects are selected from the group consisting of skin conditions such as erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis, mucous membrane conditions such as oral mucositis and proctitis, hair follicle conditions such as alopecia, cytotoxicity and polynucleic acid damage.

[0019] Additional embodiments include methods of treating a patient including topically applying a sufficient amount of nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent, evaporating solvent, and applying radiotherapy to the patient. In certain embodiments, the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

[0020] Further embodiments include methods of treating a patient, including topically applying a sufficient amount of nitroxide radioprotector to prevent or treat harmful side effects caused by radiotherapy, wherein the nitroxide radioprotector is in solution and is in the form of a low-residue gel or thickened liquid. In certain embodiments the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

Brief Description of the Drawings

[0021] Figure 1 is a bar graph providing the measured concentration of normal Tempol in receptor fluid after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0022] Figure 2 is a bar graph providing the measured concentration of oxidized Tempol oxidized receptor fluid after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0023] Figure 3 is a bar graph providing the measured concentration of normal Tempol in wipe samples after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0024] Figure 4 is a bar graph providing the measured concentration of oxidized Tempol in wipe samples after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0025] Figure 5 is a line graph comparing the measured concentration of normal and oxidized Tempol in tape strips after *in vitro* percutaneous absorption of Formulation I into human skin for 15 minutes.

[0026] Figure 6 is a line graph comparing the measured concentration of normal and oxidized Tempol in tape strips after *in vitro* percutaneous absorption of Formulation II into human skin for 15 minutes.

[0027] Figure 7 is a line graph comparing the measured concentration of normal and oxidized Tempol in tape strips after *in vitro* percutaneous absorption of Formulation III into human skin for 15 minutes.

[0028] Figure 8 is a line graph comparing the measured concentration of normal and oxidized Tempol in tape strips after *in vitro* percutaneous absorption of Formulation IV into human skin for 15 minutes.

[0029] Figure 9 is a bar graph providing the measured concentration of normal Tempol in tape strips after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0030] Figure 10 is a bar graph providing the measured concentration of oxidized Tempol in tape strips after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0031] Figure 11 is a bar graph providing the measured concentration of normal Tempol on viable epidermis and dermis after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0032] Figure 12 is a bar graph providing the measured concentration of oxidized Tempol on viable epidermis and dermis after *in vitro* percutaneous absorption of four different topical Tempol formulations (Formulations I-IV) into human skin for 15 minutes.

[0033] Figure 13 is a bar graph providing the measured concentration of normal Tempol in receptor fluid after *in vitro* percutaneous absorption of a moderately gelled 7% Tempol ethanol/water topical formulation into human skin for 15 minutes.

[0034] Figure 14 is a bar graph providing the measured concentration of oxidized Tempol in receptor fluid after *in vitro* percutaneous absorption of a moderately gelled 7% Tempol ethanol/water topical formulation into human skin for 15 minutes.

Detailed Description

Radiotherapy and Cancer

[0035] Radiation therapy works by directing ionizing radiation into the area being treated with the goal of damaging the genetic material of cancerous cells thereby making it impossible for these cells to divide. Accordingly, radiotherapy is an important tool in the fight against cancer and is used in the treatment of as many as 50% of all cancer patients. In fact, more than half a million cancer patients receive radiation therapy each year, either alone or in conjunction with surgery, chemotherapy or other forms of cancer therapy. Other terms for radiotherapy include radiation therapy, x-ray therapy, electron beam therapy, cobalt therapy, or irradiation.

[0036] Radiotherapy is especially useful in cases where surgical removal of the cancer is not possible, where surgery might debilitate the patient, or where surgical debulking of the tumor has not absolutely removed all cancerous tissue. Radiotherapy is routinely used following surgery to destroy any cancer cells that were not removed by surgery. Further uses of radiotherapy are prior to surgery where it can "shrink" a previously inoperable tumor down to a manageable size to enable surgical excision.

[0037] Radiation therapy can also be used to help relieve symptoms of advanced cancer (such as bleeding or pain), even if a cure is not possible. Over one-third of the practice of radiation therapy is palliative. The typical intent of palliative treatment is to relieve pain quickly and maintain symptom control for the duration of the patient's life. Accordingly, treatment is usually tailored to the patient's clinical condition and overall prognosis. Palliative treatment is often complementary to analgesic drug therapies and may enhance their effectiveness because it can directly target the cause of pain.

[0038] Specifically, radiotherapy can be used to treat localized solid tumors, such as cancers of the skin, head and neck, brain, breast, prostate, cervix, and the like. Radiation therapy can also be used to treat cancers of the blood-forming cells and lymphatic system including leukemia and lymphoma respectively, and the like. Mucous membranes or hair in the vicinity of the radiation or in the path of the radiation (e.g., scalp hair in the case of a brain tumor and rectal mucosa in the case of prostate cancer) can be protected using the present invention.

Radiation Forms and Dosage

[0039] External beam radiation therapy commonly uses photons, which are sometimes called "packets of energy," to treat cancer. It is an object herein to ameliorate the negative effects of all radiotherapy regardless of the form of the photon or particle, including x-rays, gamma rays, UV rays including UV-A, UV-B and UV-C, neutrons, protons, and electrons including beta particles and the like.

[0040] X-rays are a very common form of radiation used in radiotherapy. Gamma rays are another form of photons used in radiotherapy. Gamma rays can be produced spontaneously as certain elements (such as radium, uranium, and cobalt 60), which release radiation as they decompose, or decay. Each element decays at a specific rate and can give off energy in the form of gamma rays and other particles. Typically x-rays and gamma rays have the same general effect on cancer cells.

[0041] External beam radiation therapy can be delivered by means of a linear accelerator. Typically, linear accelerators use powerful generators to create the high energy rays for external beam radiation therapy. Generally, linear accelerators are capable of producing x-rays at various energies. The linear accelerator can include a special set of lead shutters, called collimators, which focus and direct the rays to the tumor. The linear accelerator can be a large "L-shaped" design which allows it to rotate and deliver radiation from all angles. Multiple angles allow the maximum amount of radiation to be delivered to the tumor while delivering a minimal amount of radiation to the surrounding healthy tissue. The formulations and methods described herein can be used in conjunction with collimators or other devices and methods that limit radiation exposure to normal cells.

[0042] Formulations and methods described herein are capable of ameliorating the effects of most forms of radiotherapy. For example, the compositions and methods can ameliorate the effects of local-field radiation and wide-field radiation. Local field radiation relates to a narrow beam of radiation directed at the specific metastatic site or sites. Customarily, local field radiation has tended to be used for patients with a long life expectancy and fewer metastatic sites. In contrast, wide-field radiation employs a larger field of radiation and is often used to treat patients with a shorter life expectancy and multiple metastatic pain-causing sites.

[0043] Radiotherapy dosage is measured by the scientific unit rad (radiation absorbed dose) which is a radiation energy dose equal to an energy of 100 ergs per gram of irradiated material. A patient who receives radiation therapy as a treatment for cancer can receive several thousand rads over a very short period of time (weeks or months). In contrast, a typical scanning x-ray contains far fewer rads. For example, modern mammography systems used to take x-ray images of the breast use approximately 0.1 to 0.2 rad dose per x-ray.

[0044] According to traditional radiotherapy, the larger the daily dose of radiation, the lower the total dose that can be administered because of limits to normal tissue tolerance. Proportionately more tumor cells are killed when the daily radiation dose is larger. Typically a balance is obtained between the killing of tumor cells and the adverse radiation effects on normal tissues, which are largely a function of the daily dose. A number of different schedules have been developed that take into account specific tumor characteristics and the tolerance of normal tissues. The literature is divided regarding the optimal radiation schedule to achieve tumor regression and disease palliation of either primary or metastatic sites. Generally, however, radiation treatment is planned in relation to clinical status. Because a main objective herein is to ameliorate the negative effects of radiation therapy, normal tissue can have a higher tolerance to radiation therapy and larger dosages of radiation can be administered safely.

Side Effects of Radiation

[0045] In general, radiation therapy is a local treatment. It typically affects the cells in the treated area. However, as mentioned above, in addition to damaging cancer cells, radiation can also damage normal cells located in the treated area. Normal cells that are located in the treated area can include skin cells, mucous membranes, hair follicles, and the like.

[0046] Radiation side effects are typically restricted to the radiation portal and can be classified as either acute, occurring during or immediately after the course of radiation therapy, or late, occurring months to years later. Acute radiation effects are more prominent with radiation schedules that deliver high total doses of radiation with small daily fractions; they generally begin at the end of the second week of therapy. Acute radiation effects, occurring primarily at skin and mucosal surfaces, usually consist of an inflammatory response such as skin erythema or pigmentation, or as mucositis. Late radiation effects may arise without any preceding acute reactions. Fibrosis is the most common type of late radiation injury and can be observed in many types of tissue, including skin.

[0047] Other skin conditions caused by radiation therapy include dry and moist desquamation. Dry desquamation, which is characterized by dry and flaky skin and pruritus in the area of irradiation. Moist desquamation, is characterized by sloughing of the epidermis, exposing the moist, raw, dermis layer of the skin.

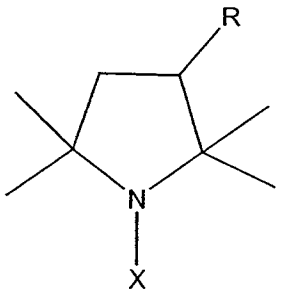
[0048] The rate at which particular hair cells grow is directly proportional to their sensitivity to radiotherapy. Accordingly, the following lists represents particular hair cells' sensitivity to radiotherapy in decreasing order: scalp hair, male beard, eyebrows axilla, pubis, and lastly fine hair. The hair follicle's epithelium is derived from the epidermis and is similarly radiosensitive. As a result, the follicular cells may develop an acute dermatitis, or hyperpigmentation earlier than other cells in the dermis. Hair follicles' sensitivity to radiation can often lead to alopecia in a patient undergoing radiotherapy.

[0049] One objective described herein is to ameliorate the negative effects of radiation therapy on normal cells, regardless of whether the effect is acute or late, or whether the effect relates to the patient's skin, mucous membranes, hair follicles, or other treated areas.

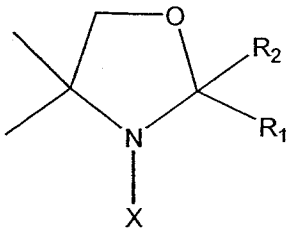
Nitroxide Radioprotectors

[0050] The term nitroxide radioprotectors, as used herein, includes any nitroxide capable of ameliorating an effect of radiotherapy. Typically nitroxides relate to stable free radical compounds that can react with a variety of biologically relevant compounds, including other free radicals, such as OH and H. Generally nitroxide radioprotectors can ameliorate most of the effects of radiotherapy including, but not limited to, protecting against cytotoxicity and polynucleic acid (e.g., DNA, RNA) damage, including mutagenicity. Further examples of effects that nitroxide radioprotectors can ameliorate include, but are not limited to skin conditions, mucous membrane conditions, and hair follicle conditions. In certain embodiments nitroxide radioprotectors include nitroxides that can react with oxy radicals, such as antioxidants, for example. In additional embodiments, nitroxide radioprotectors can neutralize superoxides and hydrogen peroxide.

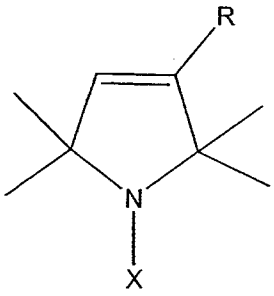
[0051] According to certain embodiments the nitroxide radioprotector can be selected from the following formulas:



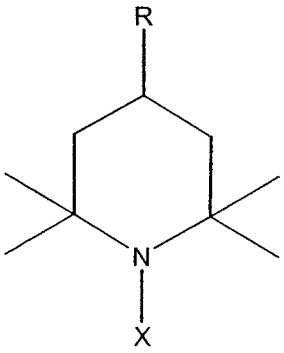
[0052] Wherein X is selected from O· and OH, and R is selected from COOH, CONH, CN, and CH_2NH_2



[0053] Wherein X is selected from O· and OH, and R_1 is selected from CH_3 and spirocylohexyl, and R_2 is selected from C_2H_5 and spirocyclohexyl



[0054] Wherein X is selected from O· and OH and R is selected from CONH.



[0055] Wherein X is selected from O· and OH and R is selected from H, OH, and NH_2 and T is selected from O.

[0056] Suitable Nitroxide radioprotectors can also be found in Proctor, US Patent No. 5,352,442, and Mitchell et al., U.S. Patent No. 5,462,946, both of which are hereby incorporated by reference in their entireties.

[0057] A non-limiting list of nitroxide radioprotectors include, 2-ethyl-2,5,5-trimethyl-3-oxazolidine-1-oxyl (OXANO), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL), 4-amino-2,2,6,6-tetramethyl-1-piperidinyloxy (Tempamine), 3-Aminomethyl-PROXYL, 3-Cyano-PROXYL, 3-Carbamoyl-PROXYL, 3-Carboxy-PROXYL, and 4-Oxo-TEMPO. These materials can be used as the sole active ingredient, or can be used with hair-growth-promoters such as Nicorandil and Minoxidil.

[0058] As used herein, nitroxide radioprotectors are solutes dissolved in a suitable solvent. This is to be distinguished from dispersions, suspensions, or emulsions of nitroxide radioprotectors, as were used in the prior art.

[0059] Although at least one nitroxide radioprotector is an active ingredient in all compositions described herein, these compositions can also include other active ingredients that are capable of ameliorating the negative effects of radiotherapy or chemotherapy. Accordingly, nitroxide radioprotectors can be used alone or in combination with other nitroxide radioprotectors, hair growth stimulants or additaments. Other hair growth stimulants and additaments include hydroxyl radical scavengers, antiandrogens and other compounds described in International Publication No. WO 87/00427 and European Patent application No. 89300785.6, both of which are hereby incorporated by reference in their entirety. In certain embodiments, nitroxide radioprotectors can be used along with other anti-oxidative agents such as glutathione and the like.

[0060] Nitroxide radioprotectors can ameliorate numerous negative effects of radiotherapy including conditions to the skin, mucous membranes, hair follicles, and the like. Skin conditions that nitroxide radioprotectors can help prevent or treat include erythema, folliculitis, fibrosis, dry desquamation moist desquamation, hyperpigmentation and dermatitis and the like. Mucous membrane conditions that nitroxide radioprotectors can help prevent or treat include oral mucositis, proctitis, and the like. Nitroxide radioprotectors can also help prevent or treat alopecia and the like by stimulating hair growth. Stimulating hair growth can include increasing rate of growth, increasing hair diameter, follicular neogenesis, and the like. Nitroxide radioprotectors can also inhibit hair loss or alopecia from progressing.

[0061] Further embodiments herein include methods of preventing or treating hair loss or alopecia regardless of whether the condition was brought about by radiation therapy or other means. For example, it is well known that hair loss or alopecia can result from genetic factors, aging, local skin conditions, systemic diseases, and chemotherapy, for example. Those with skill in the art will recognize that the embodiments described herein encompass compositions and methods relating to formulations that are effective at treating or preventing any type of hair loss without leaving an unwanted residue on the treated area. Further embodiments include compositions and methods relating to formulations that are effective at treating or preventing any type of hair loss and have a sufficient viscosity such that the formulation does not immediately run off the treated area upon application to a patient.

[0062] Developing low-residue formulations can be done by preparing solutions of nitroxide radioprotectors in low-residue gels, thickened liquids, liquids and the like. Developing low-residue formulations with sufficient viscosity can be done by preparing solutions of nitroxide radioprotectors in low-residue gels or thickened liquids.

[0063] In certain embodiments, the nitroxide radioprotector is present in a topical solution at between approximately 5-15% by weight. In other embodiments, the nitroxide radioprotector is present in a topical solution at between approximately 7-12% by weight. In more specific embodiments, the nitroxide radioprotector makes up 7% by weight of the topical solution. Preferably the nitroxide radioprotector is dissolved in an ethanol based solution.

[0064] A gel according to the present invention will typically comprise a major amount of a liquid phase and a minor amount of a thickening or gelling agent. The gelling agent, in preferred embodiments, will comprise only 5%, 4%, 3%, 2%, 1%, 0.5% or less of the total volume or weight of the composition; thus, when applied to the skin or mucosa, the liquid can evaporate, leaving only the gelling agent and the active ingredient. In this manner, 98%, 99%, or more of the carrier for the drug can disappear prior to radiotherapy, greatly reducing or eliminating topical burning due to the bolus effect.

[0065] It should be noted that in a preferred embodiment of the invention, the liquid phase of a rectal gel (or other gel for mucosal use) is specifically selected for non-irritating mucosal properties. Thus, an aqueous vehicle is appropriate, as well as non-

irritating alcohols (such as glycols or polyols) and other non-irritating solvents. It may be desirable, in practicing the present invention, to rectally administer an effective, radioprotective quantity of a nitroxide gel, and then preferably to retain the gel in the rectum during radiotherapy, or less preferably to remove the gel prior to radiotherapy.

Tempol

[0066] As mentioned above, one preferred nitroxide radioprotector that can be used in the pharmaceutical formulations described herein is Tempol. Tempol is a stable nitroxide radical which is readily available from commercial suppliers. Tempol is characterized by the chemical formula 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

[0067] Tempol can ameliorate numerous negative effects of radiotherapy including conditions to the skin, mucous membranes, hair follicles, and the like. Skin conditions that Tempol can help prevent or treat include erythema, folliculitis, fibrosis, dry desquamation moist desquamation, hyperpigmentation, dermatitis, and the like. Mucous membrane conditions that Tempol can help prevent or treat include oral mucositis, proctitis, and the like. Hair follicle conditions that Tempol can help prevent or treat include alopecia and the like by stimulating hair growth. Stimulating hair growth relates to increasing rate of growth, increasing hair diameter, follicular neogenesis and the like. Tempol is also capable of inhibiting hair loss or alopecia from progressing.

[0068] As mentioned in the Background, the prior art has limited the topical use of Tempol to the formulations selected from creams, lotions, shampoos, cream rinses, and ointments. This invention focuses on the discovery that prior art topical forms of Tempol should not be administered shortly before the actual delivery of radiotherapy to the patient. These prior art topical formulations leave a residue or film on the patient's treated area (e.g., skin, mucous membranes). If this residue or film is left on the treated area before radiotherapy, it can intensify or absorb the radiation and can cause potentially severe burning. This burning caused by the residue or film can be described as a bolus effect. (See generally, Hilderley, *Oncology Nursing Forum*, vol. 10 No. 1, pp.51-56 (1983)) Accordingly, compositions and methods herein include topical formulations that can be administered to a patient shortly before the actual delivery of radiotherapy. This can be done by topically

applying Tempol in the form of a low-residue formulation, including, but not limited to solutions of Tempol in low-residue gels, thickened liquids, liquids and the like.

Suitable Solvents

[0069] Nitroxide radioprotectors, such as Tempol, are readably soluble in aqueous solutions. In some embodiments nitroxide radioprotectors can be dissolved in a solvent and prepared into a formulation including low-residue gels, low-residue thickened liquids, and low-residue liquids. Those skilled in the art will readily appreciate that any water miscible liquid, at appropriate levels, can be used as a solvent, including, but not limited to, glycerin, PEG's, polysorbates, etc. Because a main objective of the formulations and methods provided herein is to prepare low-residue nitroxide radioprotector formulations, embodiments herein include solvents that are relatively volatile. The term "relatively volatile" relates to solvents that are readily vaporizable at relatively low temperatures. For example, embodiments herein include solvents that are readily vaporizable between about 0-38° C. Such liquids, for example, may advantageously have a vapor pressure of at least 50mmHg at 25° C, and more preferably a vapor pressure of at least 75, 90, 100, 150, 200, 250, or 300 mmHg. Accordingly, further embodiments include formulations and methods wherein the solvent has completely or substantially evaporated prior to the application of radiotherapy to the treated area.

[0070] The following is a non-exclusive list of solvents that can be used as a solvent for nitroxide radioprotectors: water, urea, alcohols and glycols. Any alcohol capable of dissolving nitroxide radioprotectors can be used in the formulations and methods described herein; examples include methanol, ethanol, propanol, butanol and the like. Likewise, any glycol capable of dissolving nitroxide radioprotectors can be used in the formulations and methods described herein; examples include ethylene glycol, propylene glycol and the like. In one preferred embodiment, the solvent not only dissolves the nitroxide radioprotector, but also facilitates transdermal delivery. Thus, transdermal-delivery-facilitating agents, particular those that disrupt or solubilize components of the stratum corneum, are particularly preferred. We have found that various alcohols, for example,

facilitate penetration of nitroxide radioprotectors into the skin. Additional embodiments include available transdermal enhancers that allow for systemic treatment of a patient.

[0071] In certain embodiments of the invention, the concentration of the active ingredient, a nitroxide radioprotector, can be at a concentration level at or near its solubility limit. For example a nitroxide radioprotector can be about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% of saturation in the solution. Embodiments also include formulations where a nitroxide radioprotector is soluble enough in the solvent to promote its release at the desired rate upon application to the treated area.

[0072] In certain embodiments, the solvent can comprise between approximately 70-90% of the solution. In other embodiments, the solvent comprises between approximately 75-86% of the solution. In more specific embodiments, the solvent comprises approximately 79% of the solution.

[0073] All of the above described solvents can be used with both the low-residue gels, thickened liquids and liquids described herein.

Characteristics of nitroxide radioprotector formulations

[0074] Embodiments herein include topical formulations containing a nitroxide radioprotector dissolved in solution. All of the solvents described above can be used in the formulations described herein.

[0075] Topical formulations can be prepared such that they can readily be applied to all areas of a patients skin, including the scalp, face, neck, chest, arms, legs, torso, back, and the like. Topical formulations can also be prepared such that they can be applied to all mucous membranes of a patient including areas of the eyes, mouth, nose, vagina, rectum, and the like. In certain embodiments it is preferred that formulations used to treat mucous membranes include water, or another non-irritating solvents. In additional embodiments, the formulations to be applied to mucous membranes lack irritating solvents such as alcohol, urea, and the like.

[0076] In topical formulations, the total quantity of a nitroxide radioprotector or other active ingredients absorbed can vary greatly based on many factors including

application area size, the frequency and vigor of application, and the viscosity or thickness of the applied vehicle. Other factors influencing drug absorption are the application site, age and condition of the skin. For example, non-keratinized, aged, broken or abraded skin will result in higher drug absorption, because these skin types are more readily penetrated by an active ingredient. Accordingly, one embodiment herein is to optimize the absorption of a nitroxide radioprotector by the treated patient while maintaining a low-residue formulation.

[0077] Because a primary objective herein is to ameliorate the negative effects of radiotherapy while not enhancing a bolus effect, topical composition embodiments should be low-residue. As used herein, the term "low-residue" refers to formulations that can be applied to a patient, shortly before undergoing radiotherapy, without leaving a residue capable of enhancing a bolus effect upon delivering radiotherapy to the treated area. Any low-residue formulation can be used according to the methods described herein. Low-residue formulations include, but are not limited to, gels, liquids, thickened liquids, and the like. Those with skill in the art can readily appreciate how to prepare low-residue gels, low-residue liquids, and low-residue thickened liquids to be used according to the methods described herein.

[0078] Other embodiments include topical formulations with sufficient viscosity such that the formulation does not immediately run off the treated area upon application. In certain embodiments the pharmaceutical composition should have a viscosity that keeps the nitroxide radioprotector and other active ingredients in contact with the treated area for a sufficient period of time to allow suitable absorption to the treated area. In some embodiments, gels and thickened liquid formulations can have a suitable viscosity such that the formulation will not immediately run off the treated area. Accordingly, methods of retaining the formulation in place are encompassed herein. As mentioned above, regardless of the composition's viscosity, there should not be a residue sufficient to produce a dangerous bolus effect when radiotherapy is applied to the treated area.

[0079] Alternative embodiments include topical formulations with low viscosity, including, but not limited to, low-residue liquids and low-residue thickened liquids. In some embodiments, liquids and thickened liquids can be applied with the aid of an applicator to allow suitable application of the nitroxide radioprotector to the treated area. Applicators can

include, but are not limited to, cloths, rags, sponges, towels, gauze, and like absorbent materials, and the combination of the applicator and the nitroxide radioprotector solution is one aspect of the methods described herein.

[0080] In addition to including a nitroxide radioprotector and a solvent, the topical compositions herein can also include polymers, colorants, antimicrobials, preservatives, antioxidants, alcohols, emollients, additional active ingredients, ingredients that enhance the permeability of the treated area, water, and other ingredients commonly used in low-residue topical formulations. Additional ingredients in the compositions herein are acceptable as long as the formulation, as a whole, remains low residue.

[0081] Those with skill in the art can readily modify the thickness of nitroxide radioprotector formulations, whether gels or liquids, with polymers. Embodiments include formulations including one or more suitable polymers with moderate to high degree of compatibility with the solvent used to dissolve the nitroxide radioprotector. In certain embodiments the polymers can be selected from ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, including modified cellulose, natural polymers including collagen, polystyrene polymers, silicone polymers, inorganic polymers, and the like.

[0082] Examples of ethylene polymers that can be used include, but are not limited to, oxidized polyethylene, polyethylene, polyethylene glycol, and the like.

[0083] Examples of acrylic polymers that can be used include, but are not limited to, acrylic esters, methacrylic esters copolymer, acrylic polymer emulsion, carbomer, ethylene acrylates, methacryiol ethyl betaine, methacrylates copolymer, octylacrylamide, acrylates, butylaminoethyl methacrylate copolymer, polyacrylamidomethylpropane sulfonic acid, polyquaternium-5, polyquaternium-6, polyquaternium-7, polyquaternium-15, and the like.

[0084] Examples of polyvinylpyrrolidones (PVPs) include, but are not limited to, polyquaternium-11, polyvinylpyrrolidone (PVP), PVP/dimethylaminoethylmethacrylate copolymers, PVP/Elcosene copolymer, PVP/ethyl methacrylate/methacrylic acid terpolymer. PVP/hexadecene copolymer, PVP/VA copolymers, styrene/PVP copolymer, and the like.

[0085] Examples of polyvinyl copolymers include, but are not limited to, ethylene vinyl acetate copolymer, PVM/MA copolymer esters, vinyl acetate/crotonic acid copolymer,

vinyl acetate/crotonic acid/methacryloxybenzophenone-1 copolymer, vinyl acetate/cotonic acid/vinyl neodecanoate copolymer, carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, PEG celluloses, polyquaternium-4, polyquaternium-10, and the like.

[0086] Examples of natural polymers include, but are not limited to, acacia, agar, alginate, carrageenan, furcelleran, gelatin, ghatti gum, glycosaminoglycans, guar gum, guar gum derivative, hydroxypropyl guar, hyaluronic acid, karaya, locust bean gum, maltodextrin, pectin, tragacanth gum, xanthan, and the like.

[0087] Examples of polystyrene polymers include, but are not limited to, sodium polystyrene sulfonate.

[0088] Examples of silicone polymers include amino bispropyl dimethicone, cyclomethicone, dimethicone, dimethicone copolyol, hexamethyldisiloxane, methicone, octadecyl dimethicone, phenyl dimethicone, stearoxy dimethicone, and the like.

[0089] Examples of inorganic polymers, include but are not limited to bentonite, modified bentonite, magnesium aluminum silicate, modified hectorite, sodium magnesium silicate, and the like.

[0090] The above listed polymers can be used in all compositions described herein. For example, the polymers can be used in low-residue gels. The polymers can also be used as thickening agents in low-residue thickened liquids.

Gels

[0091] As discussed above, in some embodiments, the pharmaceutical composition is a topical formulation in the form of a low-residue gel. As used herein, a gel relates to a semisolid system of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Generally, if left undisturbed for some time, gels may be in a semisolid or gelatinous state. With some gels, small amounts of water may separate on standing.

[0092] Those with skill in the art will readily know how to prepare low-residue gels. Detail on how to prepare such gels is provided in Remington's Pharmaceutical Sciences, 18th ed. 1990, which is hereby incorporated by reference in its entirety. In one

embodiment a gel can be prepared by slowly dispersing one or more suitable polymers in the requisite amount of suitable solvents. A discussion of suitable solvents and polymers is provided above. According to one method of preparation, a polymer and a solvent can be stirred until the polymer is completely dissolved. Water can be added to the polymer/solvent solution as it is being stirred. A sufficient amount of a nitroxide radioprotector can be added to the stirred mixture until the nitroxide radioprotector is adequately dissolved.

[0093] Gels can be one-phase or multiple phase systems. A gel mass consisting of a network of small discrete particles is generally termed a two-phase system while singe-phase gels typically consist of organic macromolecules distributed uniformly throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid.

[0094] In certain embodiments, the low-residue gel can be a hydroalcoholic gel. In some embodiments an alcohol such as ethanol can be used to dissolve the nitroxide radioprotector while avoiding the use of solubilizers such as PEG-40, hydrogenated castor oil, polysorbate 20 or similar ingredients. The absence of these solubilizers can greatly improve the cosmetic feel of the product as the stickiness and rubbery feel can be virtually absent. In embodiments where the pharmaceutical composition has a significant alcohol (e.g., ethanol) content, additional preservation may not be required.

[0095] Those with skill in the art can use numerous methods to readily prepare hydroalcohol gels with the formulation characteristics described herein. According to one method of preparing hydroalcohol gels, a solution can be prepared by dissolving the nitroxide radioprotector in ethanol. The nitroxide radioprotector/ethanol solution can be added to a hydrogel. According to certain embodiments, the nitroxide radioprotector/ethanol solution can be added to a premade hydrogel using a slow moving anchor mixer, which can reduce the creation of air bubbles in the hydroalcohol gel.

[0096] Due to reduced hydrogen bonding, the viscosity of a hydroalcoholic gel is generally lower than the viscosity of a corresponding hydrogel. Regardless those with skill in the art can adjust the ingredients of the hydroalcoholic gel to prepare a composition with a suitable viscosity tailored to the desired characteristics. For example the use of the

thickening agents or polymers discussed above can be used to raise the viscosity of a particular formulation.

[0097] In some embodiments the low-residue gel can be sprayable. Methods of preparing sprayable gels are well known in the art. According to one embodiment of preparing a sprayable gel, a suitable polymer can be added to water. Upon hydration and development of structure, the thickened polymer/water mixture can be added to a nitroxide radioprotector/solvent solution.

Liquid Formulations

[0098] Further embodiments herein include nitroxide radioprotector-containing liquid formulations. For example, a nitroxide radioprotector can be dissolved in any of the suitable solvents discussed above. The following is a non-exclusive list of solvents that can be used as a solvent for Tempol: water, urea, alcohols, glycols and the like. These liquid formulations can be used with the aid of an applicator such as a towel, cloth, rag, sponge, gauze or like absorbent material in order to apply the formulation to a patient in need.

[0099] Further embodiments include adding polymers to thicken nitroxide radioprotector containing liquid solutions. Any of the above described polymers can be used as a thickener for these formulations. For example, the following polymers can be used as thickening agents ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, natural polymers, polystyrene polymers, silicone polymers, inorganic polymers, and the like.

[0100] Those with skill in the art will readily know how to prepare thickened liquid solutions according to the methods described herein. Detail on how to prepare such liquids is provided in Remington's Pharmaceutical Sciences, 18th ed. 1990, which is hereby incorporated by reference in its entirety.

[0101] When the invention is practiced with a thickened liquid, it is advantageous to thicken the liquid to a viscosity of 20-100,000 or more centipoise. In certain embodiments the formulations provided herein can have a viscosity between 400-2000 cps, or even more specific between 900-1500 cps. In more particular embodiments, the formulations can have a viscosity of approximately 1215 cps.

Methods of Using Compositions

[0102] Method embodiments include using any of the low-residue formulations described herein on a patient undergoing radiotherapy. In some embodiments the formulation can be applied shortly before radiotherapy. Suitable areas for applying the low-residue formulation include all areas of the skin and mucous membranes. Methods include, but are not limited to, applying formulations to the scalp, face, neck, chest, arms, legs, torso, back, and the like. Further methods include, but are not limited to, applying the formulations to mucous membranes, including but not limited to, areas of the mouth, nose, eyes, vagina, rectum and the like.

[0103] Some embodiments include rubbing a low-residue nitroxide radioprotector containing formulation onto an area of a patient undergoing radiotherapy. Rubbing can be accomplished using the practitioner's hands, typically gloved, or may alternatively be done with an applicator such as a cloth, towel, sponge, rag, gauze and the like. Other embodiments include spraying the low-residue formulation onto a treated area of a patient undergoing radiotherapy. Upon being sprayed on the treated area, the formulation may be left alone to absorb, or may be rubbed in to facilitate the absorption of the nitroxide radioprotector.

[0104] Further embodiments include topically applying a sufficient amount of a nitroxide radioprotector such as 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl to prevent or treat harmful side effects caused by radiotherapy, wherein the 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl is in solution and is in the form of a low-residue gel or thickened liquid.

[0105] In some embodiments, the formulations and methods described herein can be used to treat or prevent negative side effects of radiotherapy selected from skin conditions, mucous membrane conditions, and hair follicle conditions. In some embodiments the methods herein can be used to treat or prevent skin conditions including erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, dermatitis and the like. Additional embodiments include methods of treating mucous membrane

conditions such as oral mucositis, proctitis, and the like. Further embodiments include methods of treating hair follicle conditions such as alopecia.

Examples

[0106] The following examples teach methods of making and using nitroxide radioprotector formulations. These examples are illustrative only and are not intended to limit the scope of the teachings herein.

Example I

Introduction

[0107] The following study was conducted to evaluate the *in vitro* percutaneous absorption of Tempol (4-hydroxy Tempo) from four vehicles using excised human skin from elective surgery. This study was conducted using procedures described in the FDA and AAPS Report of the Workshop on Principles and Practices of In Vitro Percutaneous Penetration Studies: Relevance to Bioavailability and Bioequivalence (*Pharm. Res.* 4:265, 1987), which is hereby incorporated by reference in its entirety.

Methods

[0108] The Tempol formulations used in this *in vitro* percutaneous absorption study were formulated by Dow Pharmaceutical Sciences, Petaluma, CA. The composition of these formulations is summarized in Table 1.

Table 1.

Tempol Formulation Composition

	Reference Ethanol Solution	Lightly Gelled Ethanol / Water	Moderately Gelled Ethanol / Water	Sprayable Ethanol / Water
Formulation ID:	I	II	III	IV
	% by wt.	% by wt.	% by wt.	% by wt.
4-Hydroxy tempo	7	7	6.1	7

Ethanol	93	76.5	79.5	33
Water	0	15.5	13.1	56.87
Klucel	0	1	1.3	0
Laponite XLG	0	0	0	3.24
Total:	100	100	100	100.11

pH of Formulations II, III, and IV were adjusted to 7 -7.5 with citric acid

[0109] As indicated in Table 1, four Tempol containing formulations were prepared. Formulation I, was a reference ethanol solution, Formulation II was lightly gelled ethanol/water solution, Formulation III was a moderately gelled ethanol/water solution, and Formulation IV was a sprayable ethanol/water gel.

[0110] Franz static diffusion cells FDC-400 (15 mm diameter orifice, O-ring joint, Crown Bio Scientific, Clinton, NJ) were mounted on 9-cell manifolds and maintained at a constant temperature of 32°C by use of recirculating water baths. These cells had an opening with a nominal area of 1.767 cm^2 and a receptor compartment with a volume ranging between 12 to 14 mL. Each diffusion cell was assembled by placing the excised human abdominal skin from a single donor dermal-side down and then a Teflon® O-ring (which rested in the groove of the receptor side, bottom half, of the diffusion cell). The donor side, top half, of the diffusion cell was then placed on top of the O-ring which rested on the skin and was held in place by a pinch clamp. The joint between the donor and receptor compartments of each cell was wrapped with PARAFILM® to prevent evaporation of the receptor solution.

[0111] Each cell was then filled with receptor solution consisting of degassed PBS with 0.1% sodium azide and 1.5% Oleth-20. Air bubbles were dispelled from under the skin. The receptor fluid was continuously stirred using a Teflon magnetic stir bar and an inoculating loop cut to ~5.0 cm from the top of the loop. The skin was allowed to equilibrate with the receptor solution for 1 hour prior to application of formulation.

[0112] A finite dose (0.1 mL/cm^2) of each formulation was applied onto the skin using a syringe. Each formulation was applied in an alternating fashion to 6 diffusion cells at 0.18 mL of formulation per cell. The diffusion cell sampling port was sealed with PARAFILM® to prevent evaporation. Following the 15-minute exposure period, the entire

contents of the receptor fluid was collected into a scintillation vial. The skin was wiped twice consecutively with a dry cotton swab, and cell caps were removed. Residual formulation was removed from the stratum corneum with multiple cellophane tape-strips until no more material was removed from the skin. The epidermis was physically separated from the dermis using tweezers. Each section of skin was placed into separate vials and labeled. All receptor, wipes, tape-strips, epidermis, and dermis samples were shipped to an analytical laboratory for analysis. Tempol content was reported as "Normal" and "Oxidized".

Results

[0113] Skin penetration of Tempol ranged from 0.003 to 0.01 percent of applied dose from the four formulations. The viable epidermis and dermis levels ranged from 0.2 to 2.8 percent of applied dose for the normal analysis and 1.1 to 6.6 percent of applied dose for the oxidized analysis. The moderately gelled ethanol / water formulation, Formulation III, exhibited the highest viable epidermis/dermis levels, 2.8% of applied dose for normal analysis and 6.6% applied dose for the oxidized analysis. The sprayable ethanol / water gel formulation, Formulation IV, obtained a result of 2.1% of applied dose for normal analysis. The reference ethanol solution, Formulation I, obtained a result of 4.4% of applied dose for the oxidized analysis. Skin deposition and penetration along with dose recovery are summarized in Tables 2 and 3. More specific results are provided in Figures 1-12.

Table 2

Percutaneous Absorption of Tempol (Normal and Oxidized)

Values are in % of Applied Dose						
Formulation		Receptor Normal (%)	Wipes Normal (%)	TapeStrips Normal (%)	Viable E/D Normal (%)	Dose Recovered
I	mean	0.01	15.02	0.27	1.52	16.82
7.00% tempol	SD	0.005	8.02	0.05	1.05	8.25
	% cv	81.00	53.40	18.94	69.22	49.03
II	mean	0.003	25.17	0.29	0.20	25.66
6.98% tempol	SD	0.004	10.11	0.10	0.18	10.25
	% cv	121.12	40.17	35.92	92.27	39.95
III	mean	0.01	35.16	0.28	2.81	38.25
6.09% tempol	SD	0.01	6.55	0.15	1.96	6.49
	% cv	78.56	18.62	54.91	69.89	16.98
IV	mean	0.003	41.54	0.14	2.11	43.80
7.00% tempol	SD	0.004	14.45	0.07	0.82	14.44
	% cv	110.22	34.79	48.66	39.11	32.97

Values are in % of Applied Dose						
Formulation		Receptor Oxidized (%)	Wipes Oxidized (%)	TapeStrips Oxidized (%)	Viable E/D Oxidized (%)	Dose Recovered
I	mean	0.01	22.96	0.35	4.37	27.69
7.00% tempol	SD	0.01	9.74	0.10	2.59	11.72
	% cv	91.52	42.43	28.14	59.23	42.33
II	mean	0.004	30.75	0.52	1.06	32.34
6.98% tempol	SD	0.004	11.42	0.18	1.11	12.05
	% cv	111.15	37.13	35.54	104.64	37.27
III	mean	0.01	82.24	0.50	6.60	44.50
6.09% tempol	SD	0.01	109.93	0.27	5.61	8.80
	% cv	78.70	133.68	55.03	85.04	19.78
IV	mean	0.004	45.95	0.26	3.34	49.54
7.00% tempol	SD	0.004	14.49	0.12	1.05	14.54
	% cv	109.70	31.54	48.20	31.61	29.34

Table 3

Percutaneous Absorption of Tempol (Normal and Oxidized)

Values are in µg

Formulation		Receptor Normal (µg)	Wipes Normal (µg)	TapeStrips Normal (µg)	Viable E/D Normal (µg)	Cumm Amount (µg)	Tempol Amount (µg)	Dose Recovered
I	mean	0.58	1511.36	27.30	153.62	1692.86	10094.00	16.82
7.00% tempol	SD	0.47	788.38	5.34	106.56	809.35	100.76	8.25
	% cv	80.93	52.16	19.55	69.37	47.81	1.00	49.03
II	mean	0.32	2618.10	29.95	20.45	2668.82	10397.87	25.66
6.98% tempol	SD	0.39	1062.63	10.64	18.89	1077.32	147.06	10.25
	% cv	121.02	40.59	35.54	92.37	40.37	1.41	39.95
III	mean	0.69	3213.88	25.12	256.44	3496.12	9125.87	38.25
6.09% tempol	SD	0.54	622.82	13.58	178.77	623.95	239.04	6.49
	% cv	78.16	19.38	54.08	69.71	17.85	2.62	16.98
IV	mean	0.39	4889.76	16.68	248.48	5155.32	11763.50	43.80
7.00% tempol	SD	0.43	1702.51	8.03	98.89	1702.76	200.73	14.44
	% cv	110.15	34.82	48.13	39.80	33.03	1.71	32.97

Values are in µg

Formulation		Receptor Oxidized (µg)	Wipes Oxidized (µg)	TapeStrips Oxidized (µg)	Viable E/D Oxidized (µg)	Cumm Amount (µg)	Tempol Amount (µg)	Dose Recovered
I	mean	0.73	2312.23	35.43	439.36	2787.75	10094.00	27.69
7.00% tempol	SD	0.66	964.49	10.22	257.30	1158.30	100.76	11.72
	% cv	91.56	41.71	28.85	58.56	41.55	1.00	42.33
II	mean	0.39	3196.10	54.03	110.24	3360.77	10397.87	32.34
6.98% tempol	SD	0.44	1191.31	19.00	115.65	1257.47	147.06	12.05
	% cv	111.16	37.27	35.17	104.91	37.42	1.41	37.27
III	mean	0.78	7359.87	45.10	606.41	8012.16	9125.87	89.34
6.09% tempol	SD	0.61	9631.31	24.44	528.16	9637.88	239.04	110.11
	% cv	78.53	130.86	54.20	87.10	120.29	2.62	123.25
IV	mean	0.45	5409.74	30.07	392.67	5832.94	11763.50	49.54
7.00% tempol	SD	0.50	1722.82	14.33	126.28	1729.43	200.73	14.54
	% cv	109.61	31.85	47.68	32.16	29.65	1.71	29.34

Conclusion

[0114] The percentage of the applied dose and amount of Tempol penetrating the skin into the receptor fluid was very low and ranged from 0.003% to 0.01% and 0.32ug/1.77cm^2 to 0.78ug/1.77cm^2 of skin respectively, following a 15 minute duration of skin exposure. This study shows that the moderately gelled ethanol / water formulation, Formulation III, achieved higher skin (epidermis/dermis) levels (2.8% of applied dose for normal analysis and 6.6% applied dose for the oxidized analysis) of Tempol but not higher skin penetration compared to the reference ethanol formulation, Formulation I. Results from this initial study suggest that Formulation III would achieve comparable or better clinical

efficacy following topical application to the head. In addition, Formulation III should have better formulation retention to the skin (low run-off) compared to Formulation I.

Example II

Introduction

[0115] This study evaluated the effect of multiple applications of a moderately gelled 7% Tempol ethanol / water formulation (Formulation V) on the *in vitro* percutaneous absorption of Tempol using similar test procedures as employed in Example I. These test procedures were consistent with the FDA and AAPS Report of the Workshop on Principles and Practices of In Vitro Percutaneous Penetration Studies: Relevance to Bioavailability and Bioequivalence (*Pharm. Res.* 4:265, 1987), which is hereby incorporated by reference in its entirety.

[0116] Test formulations used in this *in vitro* percutaneous absorption study were prepared by Dow Pharmaceutical Sciences, Petaluma, CA. Formulation compositions are summarized in Table 4 The viscosity of Formulation V was measured using a Brookfield RVDV-1+ viscometer. A sample weighing 8.4134 grams had a measured viscosity of 1215 cps at 22.9°C.

Table 4

Tempol Formulation Composition

	Tempol Formulation	Vehicle Formulation	Supplier
Formulation ID:	Formulation V	Formulation VI	
	% by wt.	% by wt.	
4-Hydroxy Tempo	7	0	Mitos
Ethanol	79.0	86.0	Spectrum
Water	13.0	13.0	McGaw
Klucel	1.0	1.0	Hercules
Total:	100	100	

[0117] Each of the four application regimens were performed on six cells: (1) a single application of Tempol formulation (Formulation V) 2) two applications of Tempol formulation (Formulation V), (3) three applications of Tempol formulation (Formulation V), and (4) one application of Tempol formulation (Formulation V) followed by one application of Vehicle formulation (Formulation VI). Each application of formulation had a 30 minute duration of exposure to the skin surface.

[0118] The skin was wiped with two dry cotton swabs after each application. Upon completion of the final application and skin wiping, the stratum corneum was removed from the skin. All samples of stratum corneum along with the remaining skin (viable epidermis / dermis), receptor fluid, and skin surface wipes collected during the study were analyzed in a laboratory for Tempol content. Tempol content was reported as "Normal", "Oxidized", and "Reduced."

Methods

[0119] Franz static diffusion cells (15 mm diameter orifice, O-ring joint, Crown Bio Scientific, Clinton, NJ) were mounted on 9-cell manifolds and maintained at a constant temperature of 32 °C by use of re-circulating water baths. These cells had an opening with a nominal area of 1.77 cm^2 and a receptor compartment with a volume ranging between 12 to 14 mL. Each diffusion cell was assembled by placing the excised human abdominal skin from a single donor dermal-side down and then a Teflon® O-ring (which rested in the groove of the donor side, top half, of the diffusion cell. The donor side (top half) of the diffusion cell was then placed on top of the O-ring resting on the skin and held in place by use of a pinch clamp. The joint between the donor and receptor compartments of each cell was wrapped with Parafilm® to prevent evaporation of the receptor solution.

[0120] Each cell was then filled with receptor solution consisting of degassed PBS with 0.1% sodium azide and 1.5% Oleth-20. Air bubbles were dispelled from under the skin. The receptor fluid was continuously stirred using a Teflon magnetic stir bar and an inoculating loop cut to ~3.0 cm from the top of the loop. The skin was allowed to equilibrate with the receptor solution for 1 hour prior to the application of formulation.

[0121] A finite dose (0.1 mL/cm^2) of formulation was applied on to the skin using a displacement pipette. The formulation was applied in an alternating fashion to 6 diffusion cells at 0.18 mL of formulation per cell. The diffusion cell sampling port was sealed with PARAFILM® to prevent evaporation. Following the 30-minute exposure period, the entire contents of the receptor fluid were collected into a scintillation vial. If appropriate, the cell was re-dosed with test formulation after removal of the previous dose using two cotton swabs wiped across the skin surface. After exposure to the last application of test formulation, the skin was wiped twice consecutively with a dry cotton swab. Cell caps were removed. Residual formulation was removed from the stratum corneum with multiple cellophane tape-strips until no more material was removed from the skin. The remaining viable epidermis/dermis was collected. All receptor, wipes, tape-strips, and viable epidermis/dermis samples were shipped to an analytical laboratory for analysis.

Results

[0122] Data was provided from the analytical lab in the form of normal and oxidized Tempol concentrations. Since reduced Tempol is not detectable by the analytical method, Tempol in the samples was oxidized such that all Tempol present was in the oxidized form. Oxidized Tempol represents the total amount of Tempol recovered. Reduced Tempol was calculated as the oxidized Tempol minus the normal Tempol.

[0123] Skin penetration of reduced Tempol ranged from 0 μg/1.77 cm^2 to 0.62 μg/1.77 cm^2 of skin following a 30 minute duration of skin exposure. The second application of Tempol and additional 30 minute exposure to the skin surface resulted in a cumulative range from 2.94 μg/1.77 cm^2 to 3.80 μg/1.77 cm^2 of skin. Application of the vehicle formulation (Formulation VI) following a dose of Tempol formulation (Formulation V) did not increase the amount of reduced Tempol penetrating the skin. The third application of Tempol and additional 30 minute exposure to the skin surface resulted in a cumulative amount of 8.8 μg reduced Tempol/1.77 cm^2 of skin.

[0124] The viable epidermis and dermis levels ranged from 153.7 μg/1.77 cm^2 to 496.5 μg/1.77 cm^2 for the normal analysis, 248.0 μg/1.77 cm^2 to 595.5 μg/1.77 cm^2 for the oxidized analysis, and 57.3 μg/1.77 cm^2 to 96.9 μg/1.77 cm^2 for the reduced result. The

highest viable epidermis/dermis levels were seen with two applications of Tempol, 184.1 µg reduced Tempol/1.77 cm^2 of skin. Skin penetration and deposition are summarized in Tables 5(a and b) and 6(a and b). Other results are provided in Figures 13 and 14.

Tables 5a & 5b: Percutaneous Absorption of Tempol in µg

Table 5a Intact		Values are in µg, n=6 cells		
Test		Viable Normal	Viable Oxidized	Viable Reduced
1	mea	156.	253.	96.9
Single	SD	84.6	113.	44.0
Form. V	%	54.2	44.8	45.4
2	mea[1]	479.	663.	184.
Two applications	SD	232.	345.	167.
Form. V	%	48.5	52.1	91.2
3	mea	425.	595.	170.
Three	SD	180.	310.	130.
Form. V	%	42.5	52.2	76.8
4	mea	153.	248.	94.3
Form. V then	SD	90.6	141.	58.0
Form .VI	%	58.9	57.2	61.5

[1] Test 2.

Table 5b Normal		Values are in µg.		
Test		30 minutes Normal (µg)	60 minutes Normal (µg)	90 minutes Normal (µg)
1	mea	3.97	n/a	n/a
Single	SD	2.25	n/a	n/a
Form. V	%	56.7	n/a	n/a
2	mea	1.93	57.7	n/a
Two applications	SD	1.11	29.4	n/a
Form. V	%	57.2	50.8	n/a
3	mea	3.48	53.8	98.7
Three	SD	1.65	55.9	56.1
Form. V	%	47.4	103.9	56.8
4	mea	2.41	21.5	n/a
Form. V then	SD	1.88	12.5	n/a
Form. VI	%	78.1	58.1	n/a

Tables 6a & 6b.

Percutaneous Absorption of Tempol in µg

Table 6a. Oxidized Values are in µg.				
Test		30 minutes Oxidized (µg)	60 minutes Oxidized (µg)	90 minutes Oxidized (µg)
1	mea	3.55	n/a	n/a
Single	SD	1.95	n/a	n/a
Form. V	%	54.9	n/a	n/a
2	mea	1.96	60.7	n/a
Two applications	SD	0.86	30.6	n/a
Form. V	%	43.6	50.3	n/a
3	mea	3.71	57.6	107.5
Three	SD	1.51	61.6	61.9
Form. V	%	40.6	106.9	57.5
4	mea	3.03	19.6	n/a
Form. V then	SD	1.53	13.8	n/a
Form. VI	%	50.5	70.4	n/a

Table 6b. Reduced Values are in µg.				
Test		30 minutes Reduced (µg)	60 minutes Reduced (µg)	90 minutes Reduced (µg)
1	mea	-	n/a	n/a
Single	SD	0.42	n/a	n/a
Form. V	%	-	n/a	n/a
2	mea	0.03	2.94	n/a
Two applications	SD	0.42	1.41	n/a
Form. V	%	1424.	47.9	n/a
3	mea	0.24	3.80	8.80
Three	SD	0.25	5.96	6.05
Form. V	%	106.5	156.7	68.7
4	mea	0.62	-	n/a
Form. V then	SD	0.87	10.0	n/a
Form. VI	%	139.1	-	n/a

[0125] Drug deposition and penetration was statistically evaluated by performing unpaired t-tests (significant differences between formulations are defined with a value of $p < 0.05$). After 30 minute duration of skin exposure, the four application regimens are statistically comparable to each other in penetration for the oxidized and normal Tempol ($p < 0.05$) with the exception the oxidized Tempol for Test 2 versus Test 3 ($p=0.033$). The amount of normal, oxidized, and reduced Tempol in Test 2 was statistically comparable to Test 3 after the second application of Tempol and additional 30 minute duration of skin exposure. Test 2 after the second application of Tempol was significantly higher than Test 4 after the application of vehicle and additional 30 minute duration of skin exposure for the normal and oxidized analysis. Test 2, 3, and 4 produced comparable levels of reduced Tempol after the second dosing and additional time. The third application results from test 3 produced levels that were twice as high as the levels in the second time point. This is suggestive of Tempol reaching a steady state of absorption.

[0126] Skin deposition of normal and oxidized Tempol after multiple applications (test 2 and 3) was significantly higher ($p < 0.05$) than a single application (test 1 and 4). Reduced Tempol was statistically comparable between all four application tests. In test 4, application of the vehicle did provide a washing-in effect, increasing the levels of normal and oxidized Tempol, but it was not as great of an effect as multiple applications of the active formulation.

Conclusion

[0127] This study shows that two sequential applications of the moderately gelled ethanol / water formulation achieved higher deposition and penetration levels of Tempol than a single application.

Example 3

Prophylactic Treatment for Brain Tumor

[0128] The moderately gelled 7% Tempol ethanol/water formulation of Example II (Formulation V) is applied twice to the scalp of a brain tumor patient prior to radiation treatment, and the solvent is allowed to evaporate. Each application of the formulation has a

30 minute duration of exposure to the scalp. Conventional radiation therapy is then administered to the tumor through the scalp. Following treatment, the patient does not experience epidermal burning, and hair loss that would otherwise result within 1-2 weeks.

[0129] Although the teachings herein have been described with reference to embodiments and examples, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the teachings herein are limited only by the following claims. All references cited herein are hereby expressly incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for use in ameliorating an effect of radiotherapy on skin, mucous membranes, or hair follicles comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

2. The pharmaceutical composition of Claim 1, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

3. The pharmaceutical composition of Claim 1, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

4. The pharmaceutical composition of Claim 3, wherein the solvent is an alcohol selected from the group consisting of methanol, ethanol, propanol, and butanol.

5. The pharmaceutical composition of Claim 3, wherein the glycol is selected from the group consisting of ethylene glycol and propylene glycol.

6. The pharmaceutical composition of Claim 1, wherein the effect of radiotherapy is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity, and polynucleic acid damage.

7. The pharmaceutical composition of Claim 6, wherein the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

8. The pharmaceutical composition of Claim 6, wherein the mucous membrane condition is selected from oral mucositis and proctitis.

9. The pharmaceutical composition of Claim 6, wherein the hair follicle condition is alopecia.

10. The pharmaceutical composition of Claim 1, wherein the effective prophylactic or therapeutic amount of a nitroxide radioprotector is an amount from about 0.01 to about 100 mg/ml of the total composition.

11. The pharmaceutical composition of Claim 1, further comprising a polymer selected from the group consisting from ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, natural polymers, polystyrene polymers, silicone polymers, and inorganic polymers.

12. The pharmaceutical composition of Claim 1, having a viscosity such that the nitroxide radioprotector will remain in contact with a treated area for a sufficient period of time to allow absorption of a pharmacologically effective amount into said treated area.

13. A pharmaceutical composition for use in ameliorating an effect of radiotherapy to skin or mucous membranes, comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel or thickened liquid that does not leave an amount of residue sufficient to enhance burning to the skin or mucous membranes when radiotherapy is applied.

14. The pharmaceutical composition of Claim 13, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

15. A pharmaceutical composition for use in preventing or treating alopecia comprising:

a solvent; and

an effective prophylactic or therapeutic amount of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

16. A method of treating a patient, comprising topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat harmful side effects caused by radiotherapy, wherein the nitroxide radioprotector is in solution in a solvent.

17. The method of Claim 16 wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

18. The method of Claim 16 wherein the solution is in the form of a low-residue gel or a thickened liquid.

19. The method of Claim 16, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

20. The method of Claim 16 where the harmful side effect is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity and polynucleic acid damage.

21. The method of Claim 20 wherein, the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

22. The method of Claim 20 wherein, the mucous membrane condition is selected from oral mucositis and proctitis.

23. The method of Claim 20, wherein the hair follicle condition is alopecia.

24. A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent;

evaporating solvent;

applying radiotherapy to said patient.

25. A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent and has a sufficient viscosity such that it is retained in place on the patient;

applying radiotherapy to said patient.

NITROXIDE RADIOPROTECTOR FORMULATIONS AND METHODS OF USE

Abstract of the Disclosure

Pharmaceutical compositions useful in preventing and treating negative side effects accompanying radiotherapy are disclosed. More particularly, new formulations that can be applied to the skin and mucous membranes of patients undergoing radiotherapy and methods of using these formulations are disclosed.

S:\DOCS\MCB\MCB-2151.DOC
092403

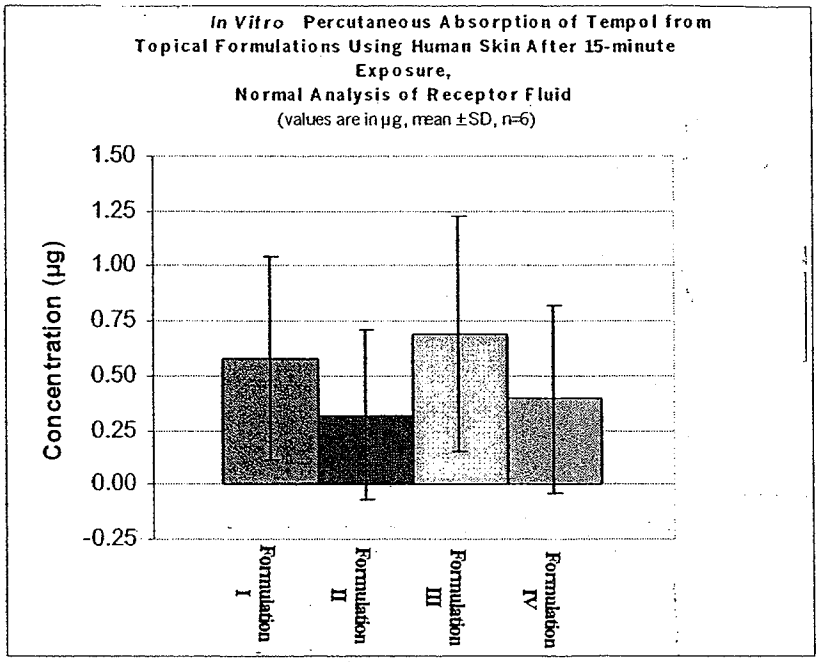

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Normal Analysis of Receptor Fluid
(values are in µg, mean ±SD, n=6)
Concentration (µg)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 1

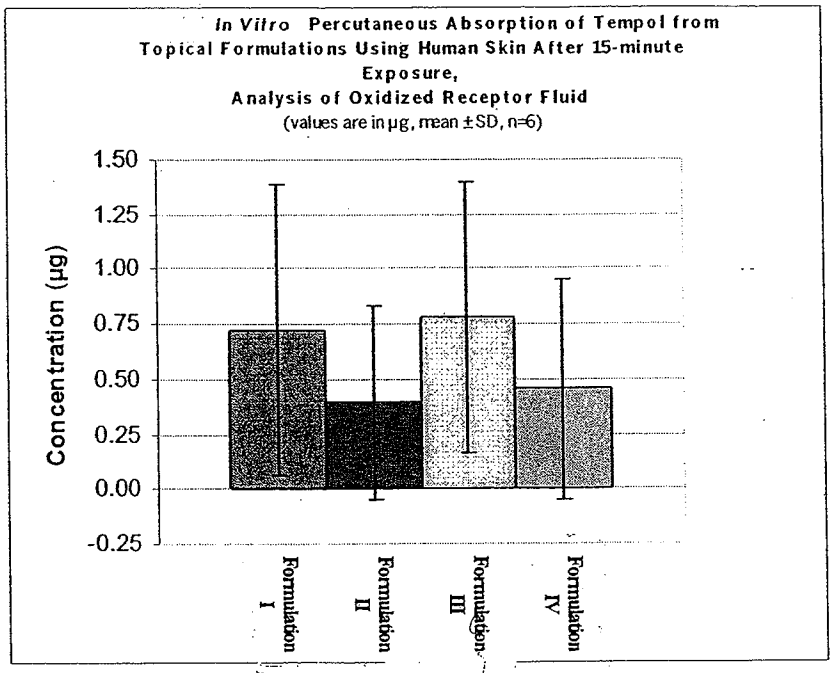

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Analysis of Oxidized Receptor Fluid
(values are in µg, mean ±SD, n=6)
Concentration (µg)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 2

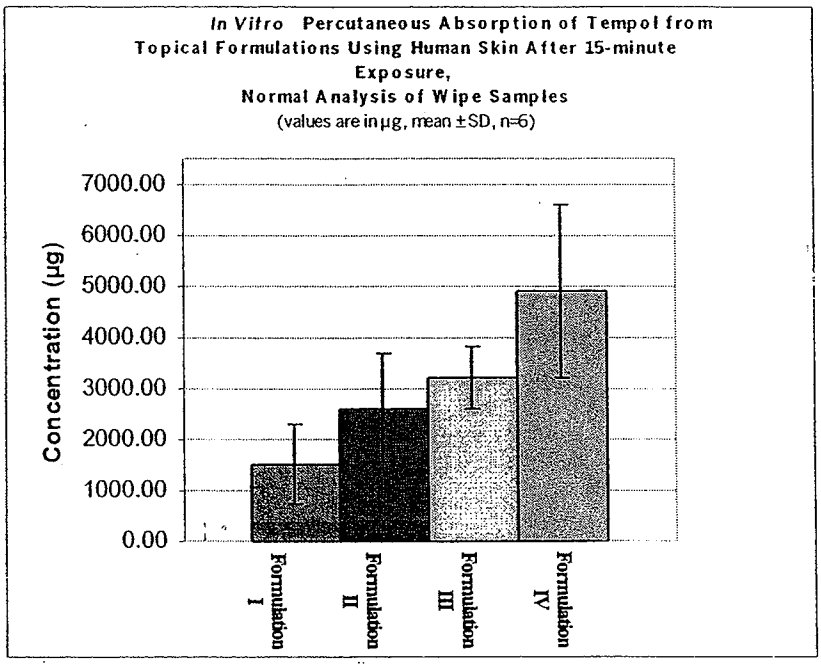

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Normal Analysis of Wipe Samples
(values are in µg, mean ±SD, n=6)
Concentration (µg)
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 3

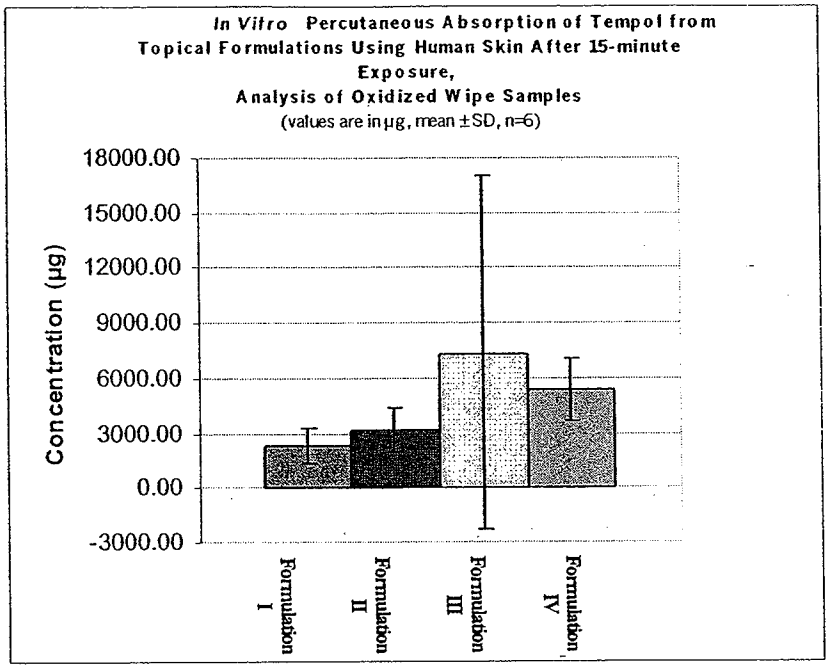

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Analysis of Oxidized Wipe Samples
(values are in µg, mean ±SD, n=6)
Concentration (µg)
18000.00
15000.00
12000.00
9000.00
6000.00
3000.00
0.00
-3000.00
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 4

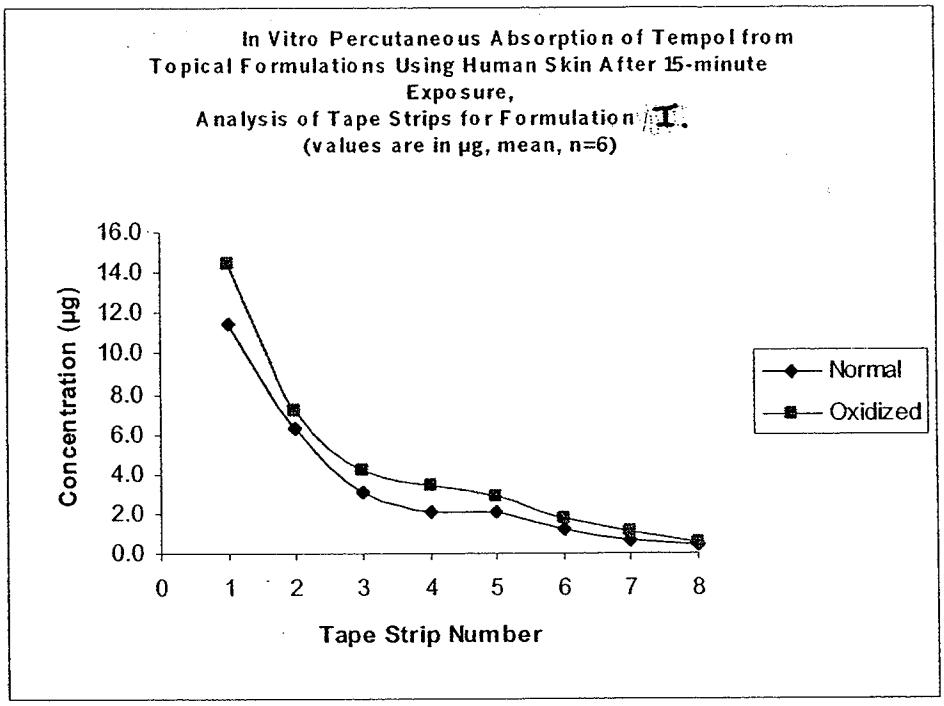

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Analysis of Tape Strips for Formulation I.
(values are in µg, mean, n=6)
Concentration (µg)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
1
2
3
4
5
6
7
8
Tape Strip Number
Normal
Oxidized

Figure 5

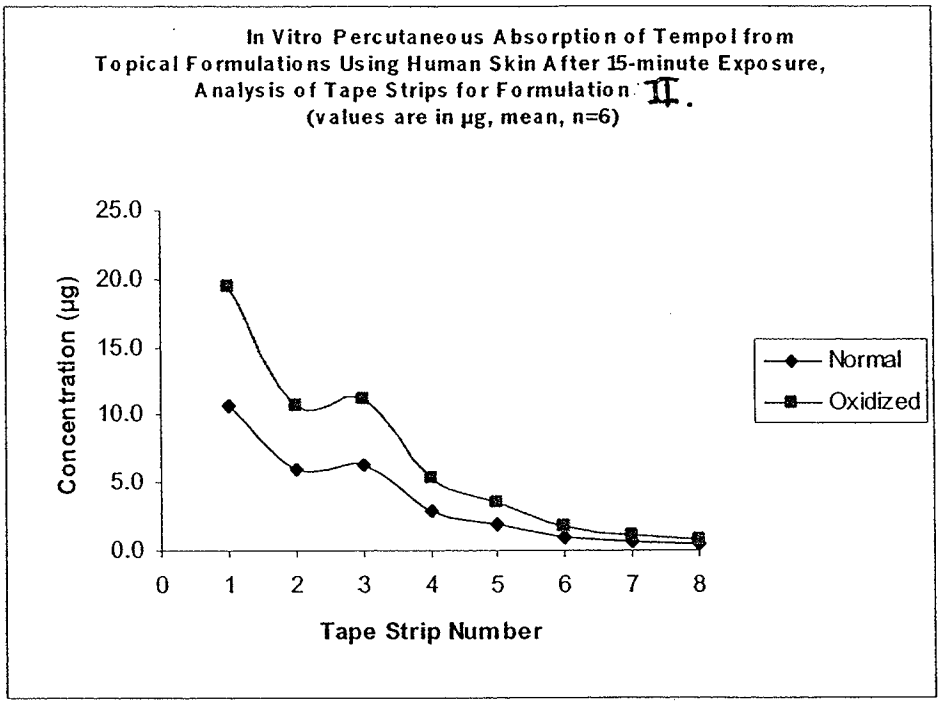

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure, Analysis of Tape Strips for Formulation II.
(values are in µg, mean, n=6)
Concentration (µg)
25.0
20.0
15.0
10.0
5.0
0.0
0
1
2
3
4
5
6
7
8
Tape Strip Number
Normal
Oxidized

Figure 6

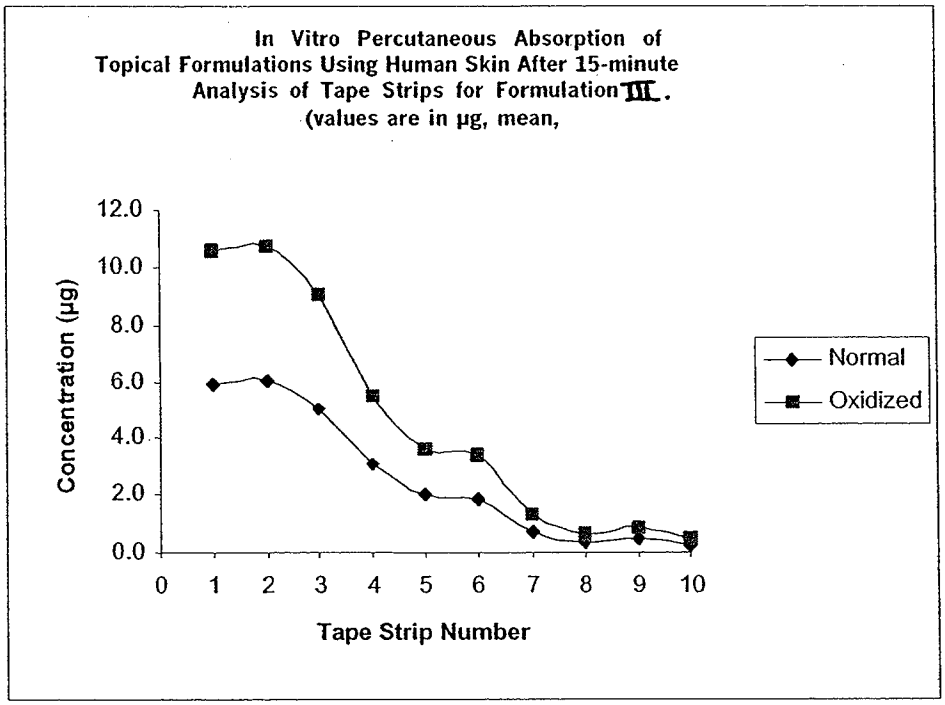

In Vitro Percutaneous Absorption of Topical Formulations Using Human Skin After 15-minute Analysis of Tape Strips for Formulation III.
(values are in µg, mean,
Concentration (µg)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0 1 2 3 4 5 6 7 8 9 10
Tape Strip Number
Normal
Oxidized

Figure 7

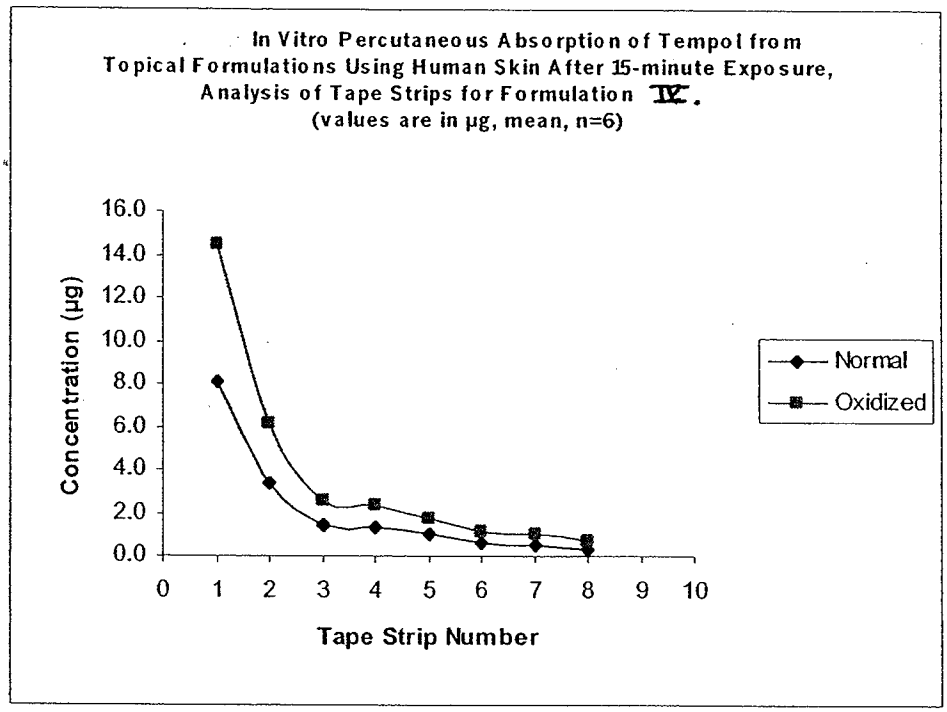

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure, Analysis of Tape Strips for Formulation IV.
(values are in µg, mean, n=6)
Concentration (µg)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0 1 2 3 4 5 6 7 8 9 10
Tape Strip Number
Normal
Oxidized

Figure 8

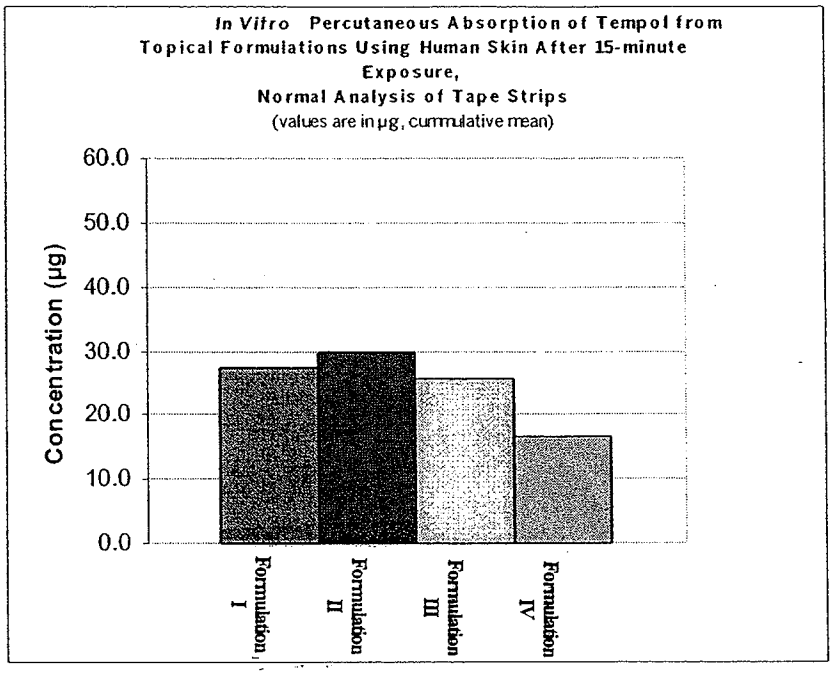

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Normal Analysis of Tape Strips
(values are in µg, cummulative mean)
Concentration (µg)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 9

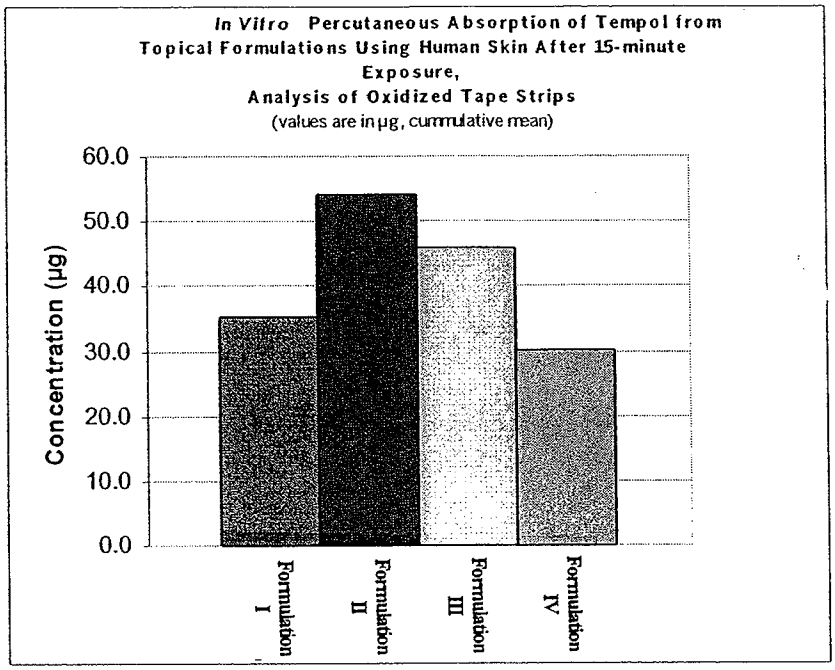

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Analysis of Oxidized Tape Strips
(values are in µg, cummulative mean)
Concentration (µg)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 10

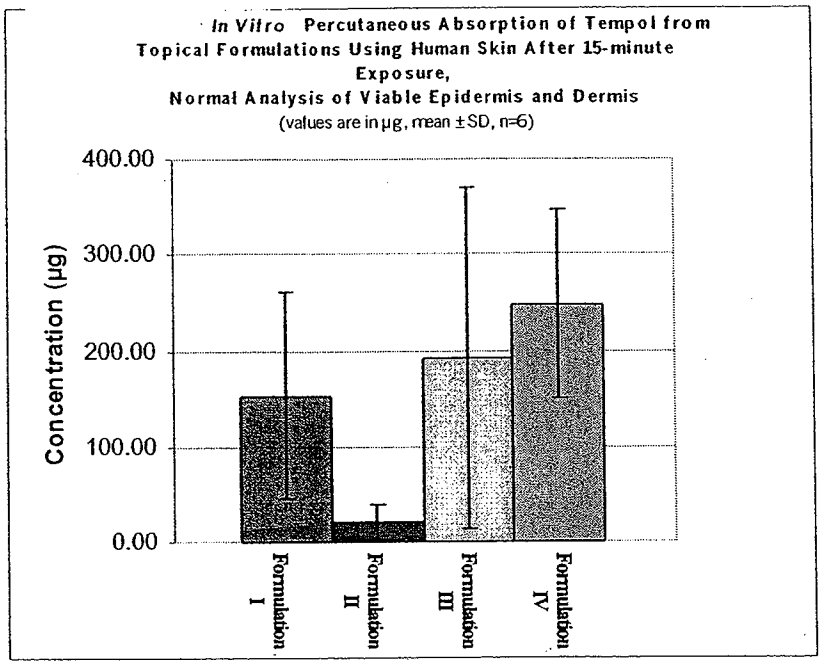

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Normal Analysis of Viable Epidermis and Dermis
(values are in µg, mean ±SD, n=6)
Concentration (µg)
400.00
300.00
200.00
100.00
0.00
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 11

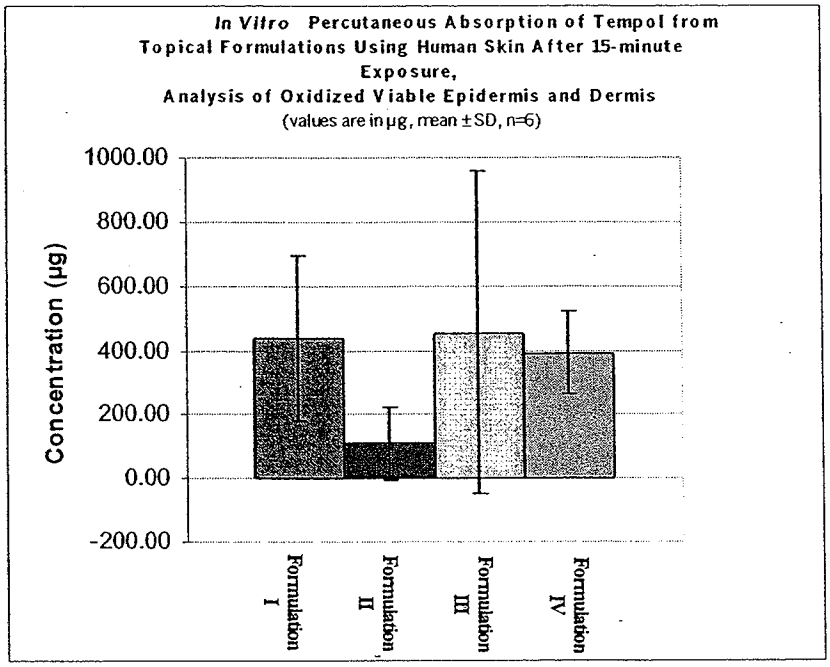

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin After 15-minute Exposure,
Analysis of Oxidized Viable Epidermis and Dermis
(values are in µg, mean ± SD, n=6)
Concentration (µg)
1000.00
800.00
600.00
400.00
200.00
0.00
-200.00
Formulation I
Formulation II
Formulation III
Formulation IV

Figure 12

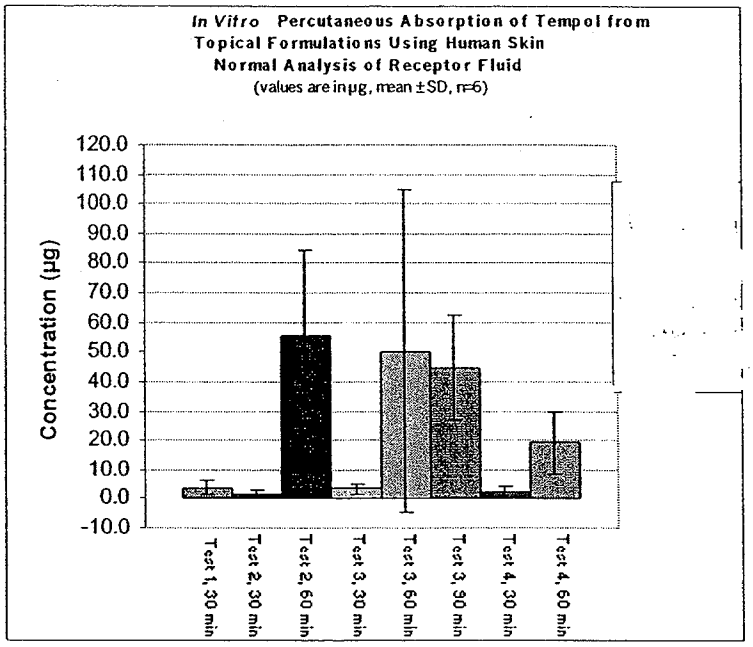

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin
Normal Analysis of Receptor Fluid
(values are in µg, mean ±SD, n=6)
Concentration (µg)
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
Test 1, 30 min
Test 2, 30 min
Test 2, 60 min
Test 3, 30 min
Test 3, 60 min
Test 3, 90 min
Test 4, 30 min
Test 4, 60 min

Figure 13

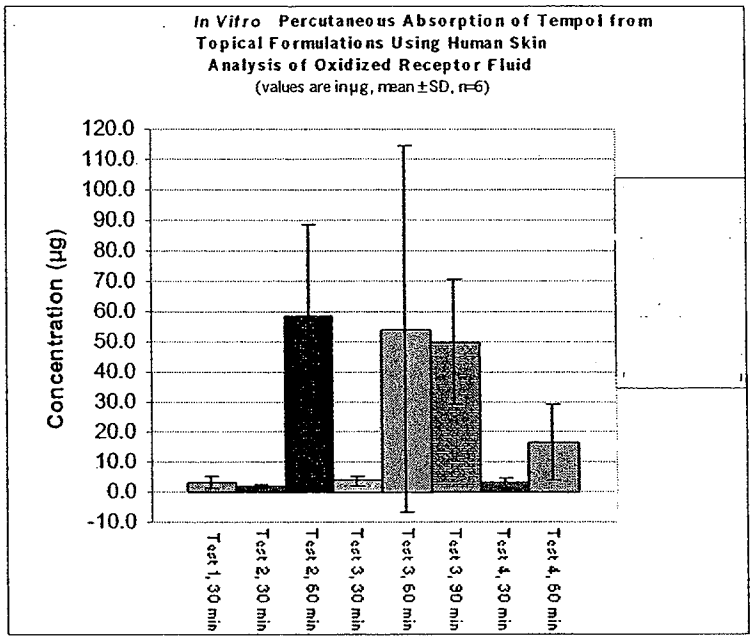

In Vitro Percutaneous Absorption of Tempol from Topical Formulations Using Human Skin
Analysis of Oxidized Receptor Fluid
(values are in µg, mean ± SD, n=6)
Concentration (µg)
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
Test 1, 30 min
Test 2, 30 min
Test 2, 60 min
Test 3, 30 min
Test 3, 60 min
Test 3, 90 min
Test 4, 30 min
Test 4, 60 min

Figure 14

NA1/CPP

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/675,225	09/29/2003	Kameron W. Maxwell	MITOS.002A	9871

20995 7590 04/11/2006

KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614

EXAMINER	
ROGERS, JAMES WILLIAM	
ART UNIT	PAPER NUMBER
1618	

DATE MAILED: 04/11/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/675,225	MAXWELL ET AL.	
	Examiner	Art Unit	
	James W. Rogers	1618	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09/29/2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *08/09/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2, 6-10,12-18,20-25 are rejected under 35 U.S.C. 102(b) as being unpatentable by Mitchell et al. (US 5,462,946).

Mitchell teaches pharmaceutical compositions and their methods of use, the compositions contain nitroxide compounds (including TEMPOL) that can be used as radiation protectants for skin, mucositous and hair loss (also known as alopecia), which can be applied as an ointment, lotion or cream (satisfying the claim for a gel or thickened liquid), and intravenously or orally by pill or lozenge. See col 1 lin 10-13, col 2 lin 53-58 and claims 1 and 10. While the patent is silent on specific solvents it is deemed inherent by the examiner that in order to make a topical cream or lotion the active ingredient would have to be dissolved in some type of solvent and the patent describes the compounds having concentrations of from 1-5 mM and the use of acceptable carriers. See col 4 lin 40-42 and lin 47-51. Regarding claims 7 and 21, while Mitchell only discloses treatment of skin conditions, it is inherent that protecting against skin conditions commonly associated with radiotherapy would include group in claims 7 and 21 (discussed as being common skin conditions in applicants spec [0004]) and since the radioprotective agent is the same in both patents it would protect against

all skin conditions the same as applicants claimed invention. Regarding claim 10, the Mitchell patent teaches the exact same amount of active ingredient for topical use as the applicant. See col 5 lin 18-21. Regarding claims 12 and 25, it is deemed inherent by the examiner and a normal part of experimentation by someone skilled in the art to adjust the viscosity of a topical gel so that it "will remain in contact with a treated area for a sufficient period of time to allow absorption of a pharmacologically effective amount into said treated area" thus this claim was given no patentable weight by the examiner. Regarding claims 24 and 25, applying the composition topically to prevent harmful effects of radiotherapy is taught by Mitchell (see col 2 lin 53-58) and evaporating solvent after applying topically is inherent since the solvents listed are volatile (methanol) and would eventually evaporate when applied to a persons skin.

Claims 1-9 and 11-25 are rejected under 35 U.S.C. 102(b) as being unpatentable by Golz-Berner et al. (US 6,426,080 B1 is used as an English equivalent to WO 99/66881).

Golz-Berner teaches a cosmetic preparation of active substances to protect the skin (including TEMPOL) in the form of a gel composed of hydrogels (including natural polymers such as hydroxymethylcellulose), solvent (including ethanol) and other ingredients such as carriers (propylene glycol and water are listed). See col 3 lin 23-29 col 6 lin 6-9, col 7 lin 3 and lin 60-62, col 9 lin 13 and lin 37-40. The examiner gave no patentable weight to the intended use phrase in claim 1 "for use in ameliorating an effect of radiotherapy on the skin, ect" because this is only an intended use and therefore has no patentable weight on the pharmaceutical composition. The examiner

gave no patentable weight to the treatment of the various skin, hair and mucous membrane conditions since the radioprotective agent (TEMPOL) is the same in both patents, it is inherent that since the claimed ingredient and the ingredient in the Golz-Berner patent are the same it would have the same effect against radiotherapy.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mitchell et al. (US 5,462,946) in view of Golz-Berner et al. (US 6,426,080 B1 is used as an English equivalent to WO 99/66881).

The Mitchell patent teaches as above.

The Mitchell patent is silent on the exact solvents and polymers used in the topical skin application.

The Golz-Berner patent teaches as above.

It would have been obvious to a person of ordinary skill in the art at the time the claimed invention was made to combine the art described in the documents above because Mitchell discloses all of the claimed invention by the applicants except the exact solvents and polymers used while Golz-Berner discloses a cosmetic preparation of active substances to protect the skin including TEMPOL and discloses the use of solvents, carriers and hydrogels which are the same as the applicants claimed ingredients (ethanol, propylene glycol, water and natural polymers). The motivation to combine the two documents would be the formulation of a pharmaceutical topical gel for use as a radioprotector, the gel composition comprised of TEMPOL, solvent and polymers.

Conclusion

No claims are allowed. Any inquiry concerning this communication or earlier communications from the examiner should be directed to James W. Rogers whose telephone number is (571) 272-7838. The examiner can normally be reached on 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Hartley can be reached on (571) 272-0616. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

MICHAEL G. HARTLEY
SUPERVISORY PATENT EXAMINER

FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. MITOS.002A	APPLICATION NO. 10/675,225
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (USE SEVERAL SHEETS IF NECESSARY)	APPLICANT Maxwell, et al.	
	FILING DATE September 29, 2003	GROUP Unknown

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)
QM		5,840,734	12/98	Berstein	X	X	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
QM		WO 00/78316 A1	12/00	PCT	X	X		

EXAMINER INITIAL		OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)

S:\DOCS\MCB\MCB-2651.DOC
042304

EXAMINER QM	DATE CONSIDERED 4-6-2006

*EXAMINER: INITIAL IF CITATION CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP 609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

Notice of References Cited	Application/Control No. 10/675,225	Applicant(s)/Patent Under Reexamination MAXWELL ET AL.	
	Examiner James W. Rogers	Art Unit 1618	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,462,946	10-1995	Mitchell et al.	514/315
*	B	US-6,426,080	07-2002	Golz-Berner et al.	424/401
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Kameron W. Maxwell
Appl. No.	:	10/675,225
Filed	:	September 29, 2003
For	:	NITROXIDE RADIOPROTECTOR FORMULATIONS AND METHODS OF USE
Examiner	:	James W. Rogers
Group Art Unit	:	1618

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

August 11, 2006
(Date)

C. Philip Poirier, Reg. No. 43,006

AMENDMENT AND RESPONSE

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is in response to the Office Action mailed April 11, 2006. The following amendments and remarks are respectfully submitted.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Original) A pharmaceutical composition for use in ameliorating an effect of radiotherapy on skin, mucous membranes, or hair follicles comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

2. (Original) The pharmaceutical composition of Claim 1, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

3. (Original) The pharmaceutical composition of Claim 1, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

4. (Original) The pharmaceutical composition of Claim 3, wherein the solvent is an alcohol selected from the group consisting of methanol, ethanol, propanol, and butanol.

5. (Original) The pharmaceutical composition of Claim 3, wherein the glycol is selected from the group consisting of ethylene glycol and propylene glycol.

6. (Original) The pharmaceutical composition of Claim 1, wherein the effect of radiotherapy is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity, and polynucleic acid damage.

7. (Original) The pharmaceutical composition of Claim 6, wherein the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

8. (Original) The pharmaceutical composition of Claim 6, wherein the mucous membrane condition is selected from oral mucositis and proctitis.

9. (Original) The pharmaceutical composition of Claim 6, wherein the hair follicle condition is alopecia.

10. (Original) The pharmaceutical composition of Claim 1, wherein the effective prophylactic or therapeutic amount of a nitroxide radioprotector is an amount from about 0.01 to about 100 mg/ml of the total composition.

11. (Currently amended) The pharmaceutical composition of Claim 1, further comprising a polymer selected from the group consisting ~~from~~of ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, natural polymers, polystyrene polymers, silicone polymers, and inorganic polymers.

12. (Original) The pharmaceutical composition of Claim 1, having a viscosity such that the nitroxide radioprotector will remain in contact with a treated area for a sufficient period of time to allow absorption of a pharmacologically effective amount into said treated area.

13. (Currently amended) A pharmaceutical composition for use in ameliorating an effect of radiotherapy to skin or mucous membranes, comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel or low-residue thickened liquid that does not leave an amount of residue sufficient to enhance burning to the skin or mucous membranes when radiotherapy is applied.

14. (Original) The pharmaceutical composition of Claim 13, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

15. (Original) A pharmaceutical composition for use in preventing or treating alopecia comprising:

a solvent; and

an effective prophylactic or therapeutic amount of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

16. (Currently amended) A method of treating a patient, comprising topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat harmful side effects caused by radiotherapy, wherein the nitroxide radioprotector is in solution in a solvent, and the solution is in the form of a low-residue gel or a low-residue thickened liquid.

17. (Original) The method of Claim 16 wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

18. (Canceled)

19. (Original) The method of Claim 16, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

20. (Original) The method of Claim 16 where the harmful side effect is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity and polynucleic acid damage.

21. (Original) The method of Claim 20 wherein, the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

22. (Original) The method of Claim 20 wherein, the mucous membrane condition is selected from oral mucositis and proctitis.

23. (Original) The method of Claim 20, wherein the hair follicle condition is alopecia.

24. (Currently amended) A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent;

evaporating solvent; and

applying radiotherapy to said patient.

25. (Currently amended) A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent, ~~and~~ has a sufficient viscosity such that it is retained in place on the patient, and the solution is in the form of a low-residue gel or a low-residue thickened liquid; and

applying radiotherapy to said patient.

REMARKS

Claims 1-25 are pending in this application. By this amendment, Claims 11, 13, 16, 24 and 25 have been amended. Claim 18 has been canceled. No new matter has been added thereby. The Examiner's rejections are traversed below.

Claim Rejections Under 35 U.S.C. § 102

The Examiner has rejected all of the pending claims under 35 U.S.C. § 102(b) as anticipated by either Mitchell et al., U.S. Patent No. 5,462,946, or Golz-Berner et al., PCT Publication No. WO 99/66881 (using U.S. Patent No. 6,426,080 as an English equivalent). Claim 18 has been canceled. Applicant traverses these rejections with respect to Claims 1-17 and 19-25.

1. Mitchell, U.S. Pat. No. 5,462,946

The Examiner has rejected Claims 1-2, 6-10, 12-18, and 20-25 under 35 U.S.C. § 102(b) as being anticipated by Mitchell et al., U.S. Patent No. 5,462,946. The Examiner characterizes Mitchell in the following terms:

> Mitchell teaches pharmaceutical compositions and their methods of use, the compositions contain nitroxide compounds (including TEMPOL) that can be used as radiation protectants for skin mucositous and hair loss (also known as alopecia), which can be applied as an ointment, lotion, or cream (satisfying the claim for a gel or thickened liquid) and intravenously or orally by pill or lozenge . . . . While the patent is silent on specific solvents, it is deemed inherent by the Examiner that in order to make a topical cream or lotion, the active ingredient would have to be dissolved in some type of solvent, and the patent describes the compound as having concentrations of from 1-5 mM and the use of acceptable carriers.

Office Action at page 2.

Independent Claims 1, 13, 15, 16, and 25 all require that the pharmaceutical composition be in the form of a "low-residue gel" or a "low-residue gel or low-residue thickened liquid." The present inventors recognized that the prior art, including the Mitchell patent cited by the Examiner, disclosed formulations that were unsuitable for administration shortly before the application of radiotherapy, as they would lead to a burning of the skin and mucous membranes in the treated area as a result of a bolus effect. *See* specification at page 2 ("These references limit the topical use of TEMPOL to formulations selected from creams, lotions, shampoos, cream rinses, and ointments. It is now recognized that these kinds of topical formulations are

unsuitable for administration shortly before the actual delivery of radiotherapy to the patient. Indeed, these product forms leave residues that can result in topical burning, including severe burns, when radiation is administered.") and page 15 ("This invention focuses on the discovery that prior art topical forms of Tempol should not be administered shortly before the actual delivery of radiotherapy to the patient. These prior art topical formulations leave a residue or film on the patient's treated area (e.g., skin, mucous membranes). If this residue or film is left on the treated area before radiotherapy, it can intensify or absorb the radiation and can cause potentially severe burning. This burning caused by the residue or film can be described as a bolus effect.").

The Mitchell reference does not contain any disclosure related to the problem of burning caused by the use of topical formulations, such as those disclosed in Mitchell, that left a residue on the patient's skin. Indeed, as noted by the Examiner, the Mitchell reference refers to topical formulations which may be "an ointment, lotion, or cream." Such formulations will, if applied shortly before the actual delivery of radiotherapy to the patient, leave a residue that will cause topical burning, as disclosed in the present specification. *See* specification at 2. Nor can the passing reference in Mitchell to a "liquid" topical formulation anticipate the "low residue gel" or "low-residue gel or low-residue thickened liquid" limitations of Claims 1, 13, 15, 16, and 25. These claims are thus not anticipated by Mitchell.

With respect to Claim 24, the Examiner states that "applying the composition topically to prevent harmful effects of radiotherapy is taught by Mitchell (*see* col. 2, lines 53-58) and evaporating solvent after applying topically is inherent since the solvents listed are volatile (methanol) and would eventually evaporate when applied to a person's skin." Office Action at page 3. Disclosure of the use a solvent may indeed inherently disclose the eventual evaporation of that solvent.

However, Claim 24 recites the steps of "evaporating solvent; and applying radiotherapy to said patient." To anticipate this claim, evaporation of the solvent in the formulation must take place before the radiotherapy is applied to the patient. Mitchell does not disclose the timing of the application of the topical formulations with respect to the application of ionizing radiation. Furthermore, as recognized by the Examiner, Mitchell is silent as to the specific solvents to be employed. *See* Office Action at page 2. It is possible that, if applied well in advance of the

application of ionizing radiation, the undisclosed solvent in the "ointment, lotion, or cream" formulation of Mitchell will evaporate before ionizing radiation is applied. However, "[i]nherency . . . may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999); *see also* M.P.E.P. § 2112(IV). Rather, "the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference." *In re Robertson*, 169 F.3d at 745. Because Mitchell does not disclose the timing of the application of the topical formulation, a disclosure of evaporation of the solvent before the application of ionizing radiation is not "necessarily present" in Mitchell. *Id.* For this reason, the Mitchell disclosure does not inherently anticipate Claim 24.

The remaining claims rejected over Mitchell depend from one of the independent claims described above, and contain all the limitations thereof. Because Mitchell does not anticipate the pending independent claims as amended, it cannot anticipate the claims depending from these independent claims. Accordingly, Claims 1-2, 6-10, 12-17, and 20-25 are not anticipated by Mitchell.

2. Golz-Berner, PCT Publication No. WO 99/66881 (U.S. Pat. No. 6,426,080)

The Examiner has also rejected Claims 1-9 and 11-25 under 35 U.S.C. § 102(b) as being anticipated by Golz-Berner et al., PCT Publication No. WO 99/66881 (employing U.S. Patent No. 6,426,080 as an English equivalent thereto). Applicant respectfully traverses this rejection.

The Examiner characterizes the Golz-Berner reference as teaching "a cosmetic preparation of active substances to protect the skin (including TEMPOL)." Office Action at p. 3. Applicant respectfully disagrees with the Examiner's characterization of the disclosure of the Golz-Berner reference. Golz-Berner discusses the use of Tempol and other nitroxides solely as test substances for determining the radical protection factor (RPF) of the cosmetic preparations disclosed. *See* Golz-Berner '080 at col. 7, lines 56-64. Applicant notes in this regard that none of the Examples in Golz-Berner disclose the presence of Tempol or any other nitroxide in the composition. *See* Golz-Berner '080 at col. 9, line 55 – col 12, line 27.

Golz-Berner thus does not disclose the use of a nitroxide radioprotector in the actual pharmaceutical composition, as set forth in the pending claims, but rather only as a test substance which may be added to the cosmetic compositions in order to determine their effectiveness.

Accordingly, Claims 1-9, 11-17, and 19-25, all of which require the presence of a nitroxide radioprotector in the claimed composition or use of a nitroxide radioprotector in the claimed method, are not anticipated by Golz-Berner.

Withdrawal of these rejections is respectfully requested.

Claim Rejection Under 35 U.S.C. § 103

The Examiner has rejected Claims 1-25 under 35 U.S.C. § 103(a) as being unpatentable over Mitchell et al., U.S. Patent No. 5,462,946, in view of Golz-Berner et al., PCT Publication No. WO 99/66881. Claim 18 has been canceled. Applicant traverses this rejection with respect to Claims 1-17 and 19-25.

Golz-Berner is concerned with the preparation of cosmetic preparations, with one stated objective of the invention being "to provide a preparation of active substances that keeps its radical protection potential over a long period of time." Golz-Berner at col. 1, lines 52-54. Thus, in contrast to the topical formulations of the present application, which are designed to leave little residue on the skin after a short period of time, Golz-Berner is concerned with maintaining the preparation on the skin for an extended period. This is shown by the ingredients in the various Golz-Berner exemplary cosmetic compositions, which are described as "creams," "sun gels" and "emulsion-based fluids." Applicant notes, for example, that each of these exemplary formulations contains a considerable amount of glycerine. Glycerine is highly hygroscopic and will slow the rate of evaporation of the solvents employed in the compositions. Because a significant amount of glycerine is included in each of the examples disclosed in Golz-Berner, topical formulations made following the teachings of Golz-Berner would not result in the "low-residue gels" or "low-residue thickened liquids" required by Claims 1, 13, 15, 16, and 25. Neither, given the presence of glycerine, would the resulting formulations meet the requirements of Claim 24, wherein evaporation of the solvent occurs before radiotherapy is applied. As a result, even if the teachings of Golz-Berner were combined with those of Mitchell, Claims 1-17 and 19-25, as presently amended, would not be rendered obvious.

Withdrawal of this rejection is respectfully requested.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 11 AUGUST 2006

By: C. Philip Poirier

C. Philip Poirier
Registration No. 43,006
Attorney of Record
Customer No. 20,995
(949) 760-0404

2818136://rc1
081006

MITOS.002A ASG
NAI/CPP

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/675,225	09/29/2003	Kameron W. Maxwell	MITOS.002A	9871

20995 7590 09/15/2006

KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614

EXAMINER	
ROGERS, JAMES WILLIAM	

ART UNIT	PAPER NUMBER
1618	

DATE MAILED: 09/15/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/675,225	MAXWELL ET AL.	
	Examiner	Art Unit	
	James W. Rogers, Ph.D.	1618	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 August 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-17 and 19-25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17 and 19-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The Amendment After Non-Final Rejection filed 08/15/2006 has been entered. Any rejections from the previous office action not addressed within this action have been withdrawn.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2, 6-10,12-18,20-25 are rejected under 35 U.S.C. 102(b) as being unpatentable by Mitchell et al. (US 5,462,946) for the reasons set forth in the office action mailed 04/11/2006.

Applicant's arguments filed 08/15/2006 have been fully considered but they are not persuasive.

Applicant asserts that Mitchell does not contain any disclosure related to the problem of burning caused by the use of topical formulations that left a residue on the patients skin. Applicant also asserts that Mitchell does not disclose a low residue gel or low-residue thickened liquid.

The relevance of this assertion is unclear. Firstly applicants claims are directed to a pharmaceutical composition in claims 1-15, if the composition disclosed is the same then the reference would anticipate applicants claimed invention, Mitchell does disclose the same pharmaceutical composition at least for claims 1-2, 6-10,12-15. Secondly for

claims 16-25 the claims are directed towards a method of treating a patient by applying a sufficient amount of a nitroxide radioprotector to prevent harmful side effects caused by radiotherapy. Mitchell teaches that the pharmaceutical composition can be used as a radiation protectant for skin, mucositous and hair loss, thereby meeting the limitations in claims 16-18 and 20-25. Mitchell does disclose the use of the pharmaceutical composition as an ointment, cream or lotion and an aerosol drop or spray which would satisfy the limitation of a low residue gel or low residue thickened liquid.

Applicant asserts that in order to anticipate claim 24 evaporation of the solvent must take place before the radiotherapy is applied to patient and Mitchell does not disclose the timing of applying the application before the radiotherapy is applied.

The examiner interpreted claim 24 in the broadest reasonable way, the claim only states that the method of treatment comprises applying a sufficient amount of nitroxide radioprotector, evaporating solvent and applying radiotherapy to said patient. The claim only states as currently written that the solvent evaporates, it does not disclose the solvent must all be evaporated. Indeed any reasonable solvent that would be used as a carrier in a pharmaceutical composition would evaporate almost immediately especially a more volatile carrier such as methanol for instance. As far as the time required to evaporate the solvent before applying radiotherapy Mitchell does not disclose this limitation but then neither does applicant, at least in claim 24. The examiner can only search the prior art for what is included within a claim limitation, since applicants gave no time for the evaporation of the solvent the examiner did not search this limitation.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mitchell et al. (US 5,462,946) in view of Golz-Berner et al. (US 6,426,080 B1 is used as an English equivalent to WO 99/66881) for the reasons set forth in the office action mailed 04/11/2006.

Applicant's arguments filed 08/15/2006 have been fully considered but they are not persuasive.

Applicant asserts that Golz-Berner is concerned with preparing cosmetic preparations, which are designed to keep its radical protection potential over a long period of time, which is in contrast to topical formulations that are designed to leave little

residue on the skin after a short time period. Applicant asserts this is because a significant amount of glycerine is included in many of the examples disclosed within and the presence of glycerin would not result in a low residue gel or low residue thickened liquid.

The relevance of these assertions is unclear. Firstly in regards to Golz-Berner being in contrast to applicants claimed invention because it keeps its radical protection potential over a long period of time. Golz-Berner was used primarily in combination with Mitchell for its disclosure of cosmetic active substances to protect the skin and the use of solvents, carriers and hydrogels, which are the same as applicants claimed ingredients (ethanol, propylene glycol, water and natural polymers), which Mitchell did not disclose. By combing the two references one skilled in the art could use the active disclosed in Mitchel (TEMPOL) with the solvents, carriers and hydrogels in Golz-Berner which are the same as applicants claimed invention and therefore would deliver TEMPOL in a low-residue gel or low-residue thickened liquid. Secondly the fact that Golz-Berner discloses compositions that comprise glycerine in the examples does not mean that Golz-Berner teaches away from applicants claimed invention since examples are always treated as non-limiting when applying prior art and there were several other carriers listed that would be volatile such as ethanol and water.

Conclusion

No claims are allowed at this time.

Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to James W. Rogers, Ph.D. whose telephone number is (571) 272-7838. The examiner can normally be reached on 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Hartley can be reached on (571) 272-0616. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

MICHAEL G. HARTLEY
SUPERVISORY PATENT EXAMINER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Kameron W. Maxwell
Appl. No.	:	10/675,225
Filed	:	September 29, 2003
For	:	NITROXIDE RADIOPROTECTOR FORMULATIONS AND METHODS OF USE
Examiner	:	James W. Rogers
Group Art Unit	:	1618

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

January 16, 2007
(Date)

C. Philip Poirier, Reg. No. 43,006

AMENDMENT AND RESPONSE

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is in response to the Office Action mailed September 15, 2006. The following amendments and remarks are respectfully submitted.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Original) A pharmaceutical composition for use in ameliorating an effect of radiotherapy on skin, mucous membranes, or hair follicles comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

2. (Original) The pharmaceutical composition of Claim 1, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

3. (Original) The pharmaceutical composition of Claim 1, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

4. (Original) The pharmaceutical composition of Claim 3, wherein the solvent is an alcohol selected from the group consisting of methanol, ethanol, propanol, and butanol.

5. (Original) The pharmaceutical composition of Claim 3, wherein the glycol is selected from the group consisting of ethylene glycol and propylene glycol.

6. (Original) The pharmaceutical composition of Claim 1, wherein the effect of radiotherapy is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity, and polynucleic acid damage.

7. (Original) The pharmaceutical composition of Claim 6, wherein the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

8. (Original) The pharmaceutical composition of Claim 6, wherein the mucous membrane condition is selected from oral mucositis and proctitis.

9. (Original) The pharmaceutical composition of Claim 6, wherein the hair follicle condition is alopecia.

10. (Original) The pharmaceutical composition of Claim 1, wherein the effective prophylactic or therapeutic amount of a nitroxide radioprotector is an amount from about 0.01 to about 100 mg/ml of the total composition.

11. (Previously presented)The pharmaceutical composition of Claim 1, further comprising a polymer selected from the group consisting of ethylene polymers, acrylic polymers, polyvinylpyrrolidones (PVPs), polyvinyl copolymers, cellulose polymers, natural polymers, polystyrene polymers, silicone polymers, and inorganic polymers.

12. (Original) The pharmaceutical composition of Claim 1, having a viscosity such that the nitroxide radioprotector will remain in contact with a treated area for a sufficient period of time to allow absorption of a pharmacologically effective amount into said treated area.

13. (Previously presented)A pharmaceutical composition for use in ameliorating an effect of radiotherapy to skin or mucous membranes, comprising:

a solvent; and

an effective prophylactic or therapeutic amount of a nitroxide radioprotector in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel or low-residue thickened liquid that does not leave an amount of residue sufficient to enhance burning to the skin or mucous membranes when radiotherapy is applied.

14. (Original) The pharmaceutical composition of Claim 13, wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

15. (Original) A pharmaceutical composition for use in preventing or treating alopecia comprising:

a solvent; and

an effective prophylactic or therapeutic amount of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl in solution in the solvent, wherein the pharmaceutical composition is in the form of a low-residue gel.

16. (Previously presented)A method of treating a patient, comprising topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat harmful side effects caused by radiotherapy, wherein the nitroxide radioprotector is in solution in a solvent, and the solution is in the form of a low-residue gel or a low-residue thickened liquid.

17. (Original) The method of Claim 16 wherein the nitroxide radioprotector is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

18. (Canceled)

19. (Original) The method of Claim 16, wherein the solvent is selected from the group consisting of water, urea, alcohols, and glycols.

20. (Original) The method of Claim 16 where the harmful side effect is selected from the group consisting of skin conditions, mucous membrane conditions, hair follicle conditions, cytotoxicity and polynucleic acid damage.

21. (Original) The method of Claim 20 wherein, the skin condition is selected from erythema, folliculitis, fibrosis, dry desquamation, moist desquamation, hyperpigmentation, and dermatitis.

22. (Original) The method of Claim 20 wherein, the mucous membrane condition is selected from oral mucositis and proctitis.

23. (Original) The method of Claim 20, wherein the hair follicle condition is alopecia.

24. (Currently amended) A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent;

evaporating sufficient solvent to substantially reduce topical burning on application of radiotherapy; and

applying radiotherapy to said patient.

25. (Previously presented) A method of treating a patient, comprising:

topically applying a sufficient amount of a nitroxide radioprotector to prevent or treat a harmful side effect caused by radiotherapy, wherein the nitroxide radioprotector is in solution in solvent, has a sufficient viscosity such that it is retained in place on the patient, and the solution is in the form of a low-residue gel or a low-residue thickened liquid; and

applying radiotherapy to said patient.

REMARKS

Claims 1-25 are pending in this application. By this amendment, Claim 24 has been amended. No new matter has been added thereby. The amendment to Claim 24 should be entered because it complies with the Examiner's suggestion that the claim should contain further limitations directed to the evaporation of the solvent. Accordingly, no showing under 37 CFR 1.116(b)(3) should be required. The Examiner's rejections are traversed below.

Claim Rejection Under 35 U.S.C. § 102

Applicant notes that the Examiner has withdrawn the previous rejection of Claims 1-9 and 11-25 under 35 U.S.C. § 102(b) as anticipated by Golz-Berner et al., PCT Publication No. WO 99/66881 (using U.S. Patent No. 6,426,080 as an English equivalent).

The Examiner has rejected Claims 1-2, 6-10, 12-18, and 20-25 under 35 U.S.C. § 102(b) as anticipated by Mitchell et al., U.S. Patent No. 5,462,946 and for the reasons set forth in the previous Office Action mailed April 11, 2006. Applicant notes that Claim 18 was canceled in the previous Amendment and Response filed August 15, 2006. Applicant traverses these rejections with respect to Claims 1-2, 6-10, 12-17, and 20-25.

The Examiner states that Mitchell discloses "the use of the pharmaceutical composition as an ointment, cream or lotion and an aerosol drop or spray which would satisfy the limitation of a low residue gel or low residue thickened liquid." The Examiner is incorrect; Mitchell does not disclose a topical formulation that satisfies either the "low-residue gel" or "low-residue thickened liquid" limitations of the pending claims.

As noted in Applicant's previous Response, the use of an ointment, cream or lotion form of a radioprotective composition shortly before the administration of radiation leaves a residue or film that leads to potentially severe topical burning. *See* specification at pp. 2, 15. This is clear from standard definitions of these terms. According to the U.S. Food and Drug Administration's Center for Drug Evaluation and Research Data Standards Manual[1], for example, an "ointment" is "[a] semisolid dosage form, usually containing <20% water and volatiles and >50% hydrocarbons, waxes, or polyols as the vehicle." Furthermore, a "cream" is "an emulsion, semisolid dosage form, usually containing > 20% water and volatiles and/or < 50% hydrocarbons, waxes, or polyols as the vehicle." Dosage forms containing such significant

[1] Available at http://www.fda.gov/cder/dsm/DRG/drg00201.htm.

amounts of residue-producing substances will not be "low-residue." Neither are "lotions" limited to low-residue forms: they are defined simply as "[a]n emulsion, liquid dosage form." Indeed, guidelines to patients undergoing radiation therapy generally counsel specifically against the use of lotions on the treated area during therapy.[2]

Nor does Mitchell disclose topical ionizing radiation protectant formulations in the form of aerosols, drops, or sprays. These forms are listed in other contexts, such as protectant compositions for use against eye diseases or as therapy for humans or plants exposed to paraquat. Even if such forms were employed as topical radiation protectant formulations, which is neither disclosed in nor suggested by Mitchell, Mitchell does not disclose that such formulations should be low-residue formulations.

Mitchell simply does not disclose "low-residue" liquids or gels. Although the relevance of this gap in the Mitchell disclosure may be "unclear" to the Examiner[3], it represents a clear legal deficiency in the pending anticipation rejection. It is black letter law that to anticipate a claim, the cited reference must teach every limitation of the rejected claims.[4] "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). Because Mitchell does not disclose the "low-residue gel" or "low-residue thickened liquid" limitations of Claims 1, 13, 15, 16, and 25, the rejection of those claims on this ground is improper and Applicant requests that it be withdrawn.

Claim 24 has been amended to require that "sufficient solvent [be evaporated] to substantially reduce topical burning on application of radiotherapy." Support for this amendment may be found, for example, at page 14, lines 21-27 of the specification. As recognized by the

---

[2] See, for example, the American Cancer Society's Detailed Guide: Breast Cancer: Radiation Therapy (available at http://www.cancer.org/docroot/CRI/content/CRI_2_4_4X_Radiation_Therapy_5.asp?sitearea=CRI&viewmode=print&) ("Lotions, powders, deodorants, and antiperspirants can interfere with external beam radiation therapy, so you should avoid using them until treatments are complete."); University of Texas MD Anderson Cancer Center, Radiotherapy: Frequently Asked Questions (available at http://www.mdanderson.org/care_centers/radiationonco/dindex.cfm?pn=D1765CAF-8E4C-11D4-80FA00508B603A14#q14) ("Do not put anything (cream, lotion, powder, makeup) on the treatment area unless your doctor or nurse says it is OK.").

[3] See Office Action mailed September 15, 2006 at 2.

[4] See M.P.E.P. § 2131.

Examiner, Mitchell is silent as to the specific solvents to be employed. *See* Office Action mailed April 11, 2006 at page 2. Mitchell does not disclose that enough of the undisclosed solvent in the "ointment, lotion, or cream" formulation will evaporate before ionizing radiation is applied to substantially reduce topical burning. Nor is such evaporation of the solvent inherent in Mitchell. "Inherency . . . may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999); *see also* M.P.E.P. § 2112(IV). Rather, "the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference." *In re Robertson*, 169 F.3d at 745. A disclosure of sufficient evaporation of the solvent before the application of ionizing radiation to avoid radiation-associated topical burning is not "necessarily present" in Mitchell. *Id.* For this reason, the Mitchell disclosure does not literally or inherently anticipate Claim 24, and Applicant requests that this rejection be withdrawn.

The remaining claims rejected over Mitchell depend from one of the independent claims described above, and contain all the limitations thereof. Because Mitchell does not anticipate the pending independent claims as amended, it cannot anticipate the claims depending from these independent claims.

Thus, none of Claims 1-2, 6-10, 12-17, and 20-25 are anticipated by Mitchell. Applicant requests that this rejection be withdrawn.

Claim Rejection Under 35 U.S.C. § 103

The Examiner has rejected Claims 1-25 under 35 U.S.C. § 103(a) as being unpatentable over Mitchell et al., U.S. Patent No. 5,462,946, in view of Golz-Berner et al., PCT Publication No. WO 99/66881. Claim 18 was canceled in the Response filed on August 15, 2006. Applicant traverses this rejection with respect to Claims 1-17 and 19-25.

The topical formulations of the present application are designed to leave little residue on the skin after a short period of time, in order to ameliorate or avoid the problem of burning caused by radiotherapy. Neither Mitchell nor Golz-Berner disclose or suggest a low-residue formulation such as that presently claimed.

As noted above, Mitchell discloses the use of a radioprotective formulation in the form of an ointment, cream, or lotion. None of these formulations satisfy the "low-residue" limitation of the claims.

The Examiner relies on Golz-Berner for "its disclosure of cosmetic active substances to protect the skin and the use of solvents, carriers, and hydrogels." When Golz-Berner is considered in its entirety, as is required, see M.P.E.P. §2141.02(VI), its disclosure would not lead one of skill in the art to a low-residue gel or low-residue thickened liquid, as the Examiner states.

Golz-Berner is concerned with the preparation of cosmetic preparations, with one stated objective of the invention being "to provide a preparation of active substances that keeps its radical protection potential over a long period of time." Golz-Berner at col. 1, lines 52-54. In its broadest disclosure, Golz-Berner teaches that the preparation achieves an incorporation of the active ingredients in an "association complex" containing not only the hydrogel components identified by the Examiner, but also a significant fraction of phospholipids (up to 30% by weight). *See* Golz-Berner at col. 2, lines 11-12; col. 3, lines 37-42. One of skill in the art, on reviewing the Golz-Berner reference for disclosure of how to prepare a topical radioprotective formulation, would also incorporate these phospholipids into the formulation to form a similar association complex with the nitroxide active ingredient. The phospholipids, which have a much higher molecular weight than the disclosed solvents, would not readily evaporate, and the resulting composition would leave a residue that would cause topical burning during radiotherapy.

Furthermore, although the Examiner has treated the examples of Golz-Berner as non-limiting, those examples must be considered for what they would suggest to one of skill in the art. See *W.L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983). As noted in the previous Response, Golz-Berner discloses exemplary cosmetic compositions, some of which are described as "creams." This term coincides with Mitchell's teaching that a "cream" form should be used for the radioprotective formulation, making it more likely that one of skill would follow the specific teachings disclosed. Each of these exemplary "cream" formulations contains not only the phospholipid-containing active complex, but also a considerable amount of glycerine, as previously noted. *See* Response filed August 15, 2006 at 9. Glycerine is highly hygroscopic and will slow the rate of evaporation of the solvents employed in the compositions. Because a

significant amount of not only phospholipid but also glycerine is included in each of the exemplary "creams" disclosed in Golz-Berner, topical formulations made following the teachings of Golz-Berner would not result in the "low-residue gels" or "low-residue thickened liquids" required by Claims 1, 13, 15, 16, and 25. Neither, given the presence of these ingredients, would the resulting formulations meet the requirements of amended Claim 24, wherein evaporation of sufficient solvent to substantially reduce the burning effect occurs before radiotherapy is applied.

As a result, even if the teachings of Golz-Berner were combined with those of Mitchell, a "low-residue" gel or thickened liquid would not result. Because this limitation, and the limitation of Claim 24 discussed above, are not found in the cited prior art references, a prima facie case of obviousness has not been established. Claims 1-17 and 19-25, as presently amended, are not obvious over the cited prior art, and withdrawal of this rejection is respectfully requested.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 16 JANUARY 2007

By: C. Philip Poirier

C. Philip Poirier
Registration No. 43,006
Attorney of Record
Customer No. 20,995
(949) 760-0404

3311184
011607

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/675,225	09/29/2003	Kameron W. Maxwell	MITOS.000GEN	9871

20995 7590 02/16/2007

KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614

EXAMINER	
ROGERS, JAMES WILLIAM	

ART UNIT	PAPER NUMBER
1618	

NOTIFICATION DATE	DELIVERY MODE
02/16/2007	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

jcartee@kmob.com
eOAPilot@kmob.com

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 10/675,225	**Applicant(s)** MAXWELL ET AL.	
	Examiner James W. Rogers, Ph.D.	**Art Unit** 1618	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 22 January 2007 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☐ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☐ The period for reply expires _____months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: *See Continuation Sheet*. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: _____.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See cont.
12. ☐ Note the attached Information Disclosure Statement(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

Continuation of 3. NOTE: Applicants newly amended claim includes the new limitation of "evaporating sufficient solvent to substantially reduce burning on application of radiotherapy. This new limitation was not present in the claim before and would require a new search by the examiner. Contrary to applicants assertion the examiner did not suggest amending the claim as now recited, rather the examiner only mentioned that the above limitation was not searched because applicant did not claim it, which was mentioned in applicants arguments filed 08/15/2006. The examiner already mentioned in the previous office action filed 09/15/2006 that the above limitation was not searched therefore the amendment was not entered .

Continuation of 11. NOTE: Applicant's assertion that Mitchell does not teach or suggest a thickened liquid or gel is not found persuasive. Thickened liquid or gel was interpreted in the broadest reasonable way by the examiner therefore the recitation of "thickened" is not considered to be very limiting. The examiner searched thickened liquid or gel to mean any composition that contained a solvent or a solution in which the solvent/solution was more viscous or thickened after addition of the ingredients, for example to make a cake one would use milk and flour, upon mixing milk with flour the batter is more thickened or viscous than just milk alone, the limitation was interpreted in a similar manner. Since an ointment, cream or lotion is thicker or more viscous than a solvent or solution on their own the limitation is considered met. As currently claimed there is no difference in the composition claims 1-2, 6-10,12-15 previously rejected. Claim 24 was not entered therefore the examiner will not address applicant's arguments for this claim.

Applicant further argues that Golz-Berner incorporated phospholipids into the formulation which would not readily evaporate. Applicants further argue that the exemplary creams contain both the phospholipids and glycerine, both of which have a slow evaporation rate. This argument is not found persuasive because Golz-Berner was used in a 103(a) rejection as a secondary reference primarily for it's disclosure of the use of solvents, carriers and hydrogels which are the same as the applicants claimed ingredients (ethanol, propylene glycol, water and natural polymers), in combination with Mitchell, who does not disclose the mandatory use of phospholipids in the nitroxide protectant composition. The rejection was based on that it would have been obvious to one skilled in the art to include and/or modify the solvents and carriers of Golz-Berner with the composition of Mitchell, especially since they are related to the same field of endeavor. The examples within Golz-berner were given solely for the purpose of illustration and were not to be construed as being limiting to their invention since many variations are possible without departing from the spirit and scope of the invention. Clearly there are other carriers that could be employed as disclosed in the specification such as ethanol and water.

MICHAEL G. HARTLEY
SUPERVISORY PATENT EXAMINER

US005462946A

United States Patent [19]

Mitchell et al.

[11] Patent Number: 5,462,946

[45] Date of Patent: Oct. 31, 1995

[54] **NITROXIDES AS PROTECTORS AGAINST OXIDATIVE STRESS**

[75] Inventors: **James B. Mitchell**, Damascus, Md.; **Amram Samuni**, Jerusalem, Islamic Rep. of Iran; **William DeGraff**, Wheaton, Md.; **Stephen Hahn**, Great Falls, Va.

[73] Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, D.C.

[21] Appl. No.: **859,622**

[22] Filed: **Mar. 20, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 494,532, Mar. 16, 1990, abandoned.

[51] **Int. Cl.**[6] **A61K 31/445**; A61K 31/505; A61K 31/52; A61K 31/44; A61K 31/425; A61K 31/415; A61K 31/40

[52] **U.S. Cl.** **514/315**; 514/256; 514/261; 514/352; 514/370; 514/377; 514/398; 514/406; 514/426; 514/427

[58] **Field of Search** 514/315, 427, 514/398, 377, 370, 406, 426, 352, 256, 261

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

8805044 7/1988 WIPO .
8805653 8/1988 WIPO .

OTHER PUBLICATIONS

Chemical Abstracts 113(7):57–854n, 1989, Rao et al. Influence of dietary riboflavin deficiency on lenticular glutathione redox cycle, lipid peroxidation, & free radical scavengers in the rat.

Chemical Abstracts 110(22):201931t, 1988, Chen et al. Florescence quenchihg of LUEUQH–type complexes by stable free radical.

E. G. Rozantsev (1970) Free Nitroxyl Radicals, Plenum Press, New York, pp. 212–216.

Chemical Abstracts (1967) vol. 66, No. 4, p. 1626, Abstract No. 16925d.

Chemical Abstracts (1991) vol. 115, No. 9, p. 379, Abstract No88347v.

Kristl et al. (1989) Drug Dev. and Industrial Pharmacy, 15(9):1423–1440.

Nilsson et al, The Journal of Biological Chemistry, vol. 264, No. 19, pp. 11131–11135 (Jul. 5, 1989).

Fridovich (1979) Oxygen Free Radicals and Tissue Damage, Ciba Foundation Symposium 65, pp. v–vi and 1–4.

Weiss et al. eds. (1988) Perspectives in Radioprotection, Pharmacology and Therapeutics 39:1–407 (Table of Contents Only).

Mitchell et al. (1987) Br. J. Cancer 55, Suppl. VIII, 96–104.

Grant et al. (1987) Grant & Hackh's Chemical Dictionary, Fifth Ed., McGraw–Hill Book Company, New York, p. 487.

Carey et al. (1977) Advanced Organic Chemistry, Part A: Structure and Mechahisms, Plenum Press, New York, pp. 467–468.

DeGraff et al. (1991) Antimutagenicity of a Low Molecular Weight Superoxide Dismutase Mimic Against Oxidative Mutagens, accepted for publication.

Gelvan et al. (1990) Free Radical Biology and Medicine, vol. 9, Suppl. 1, p. 153, Abstract 16.11.

Reddan et al. (1990) Free Radical Biology and Medicine, vol. 9, Suppl. 1, p. 20, Abstract 2.46.

Fridovich, Archives of Biochemistry and Biophysics, vol. 247, No. 1, pp. 1–11, 1986.

McCord, The New England Journal of Medicine, vol. 312, No. 3, pp. 159–163, 1985.

Samuni et al., Free Radical Biology & Medicine, vol. 6, pp. 141–148, 1989.

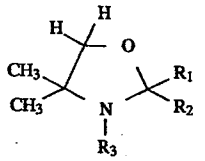
Samuni et al., The Journal of Biological Chemistry, vol. 263, No. 34, pp. 17921–17924, 1988.

Weiss, Acta Physiol Scand, Suppl. 548, pp. 9–37, 1986.

Primary Examiner—Raymond Henley, III
Assistant Examiner—William R. A. Jarvis
Attorney, Agent, or Firm—Birch, Stewart, Kolasch & Birch

[57] **ABSTRACT**

The instant invention is directed to the use of a biologically compatible composition, containing an effective amount of a metal independent nitroxide compound which is preferably represented by the formula



wherein R_1 is $—CH_3$; R_2 is $—C_2H_5$, $—C_3H_7$, $—C_4H_9$, $—C_5H_{11}$, $—C_6H_{13}$, $—CH_2—CH(CH_3)_2$, $—CHCH_3C_2H_5$, or $—(CH_2)_7—CH_3$, or wherein R_1 and R_2 together form spirocyclopentane, spirocyclohexane, spirocycloheptane, spirocyclooctane, 5-cholestane, or norbornane, R_3 is —O. or —OH, or a physiologically acceptable salt thereof, and a pharmaceutically acceptable carrier, as antioxidants capable of protecting cells, tissues, organs, and whole organisms against the deleterious effects of harmful oxygen-derived species generated during oxidative stress.

34 Claims, 9 Drawing Sheets

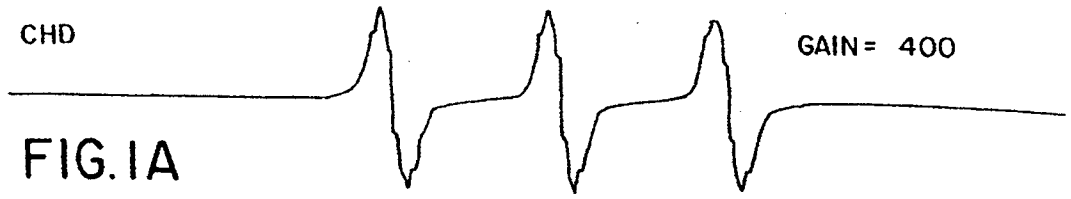


FIG. 1A

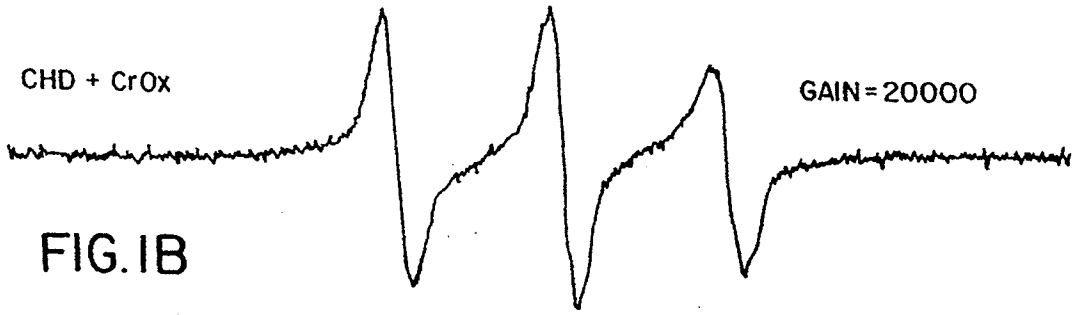


FIG. 1B

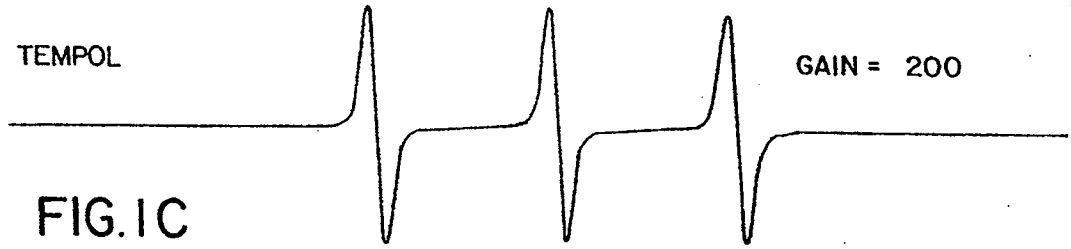


FIG. 1C

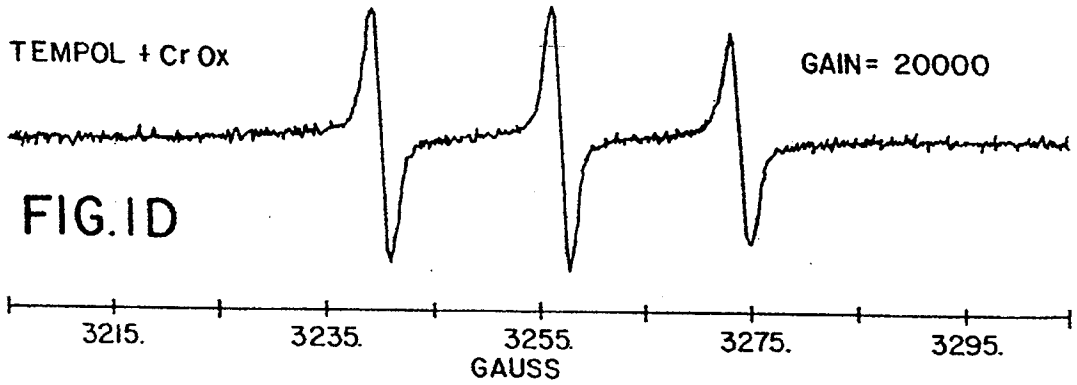


FIG. 1D

FIG. 1E

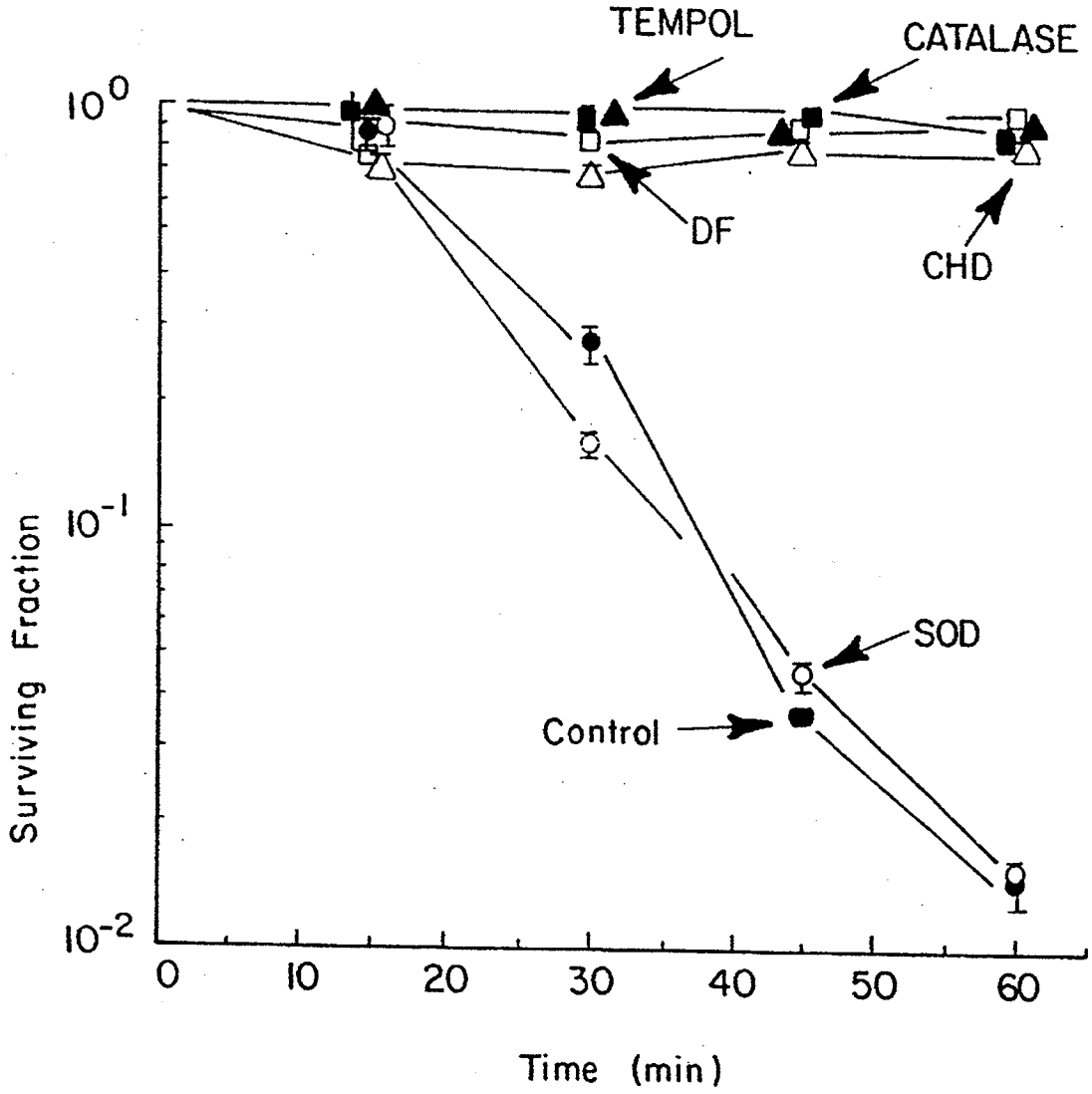

TEMPOL
CATALASE
DF
CHD
SOD
Control
10^0
10^{-1}
10^{-2}
Surviving Fraction
0
10
20
30
40
50
60
Time (min)

FIG. 2

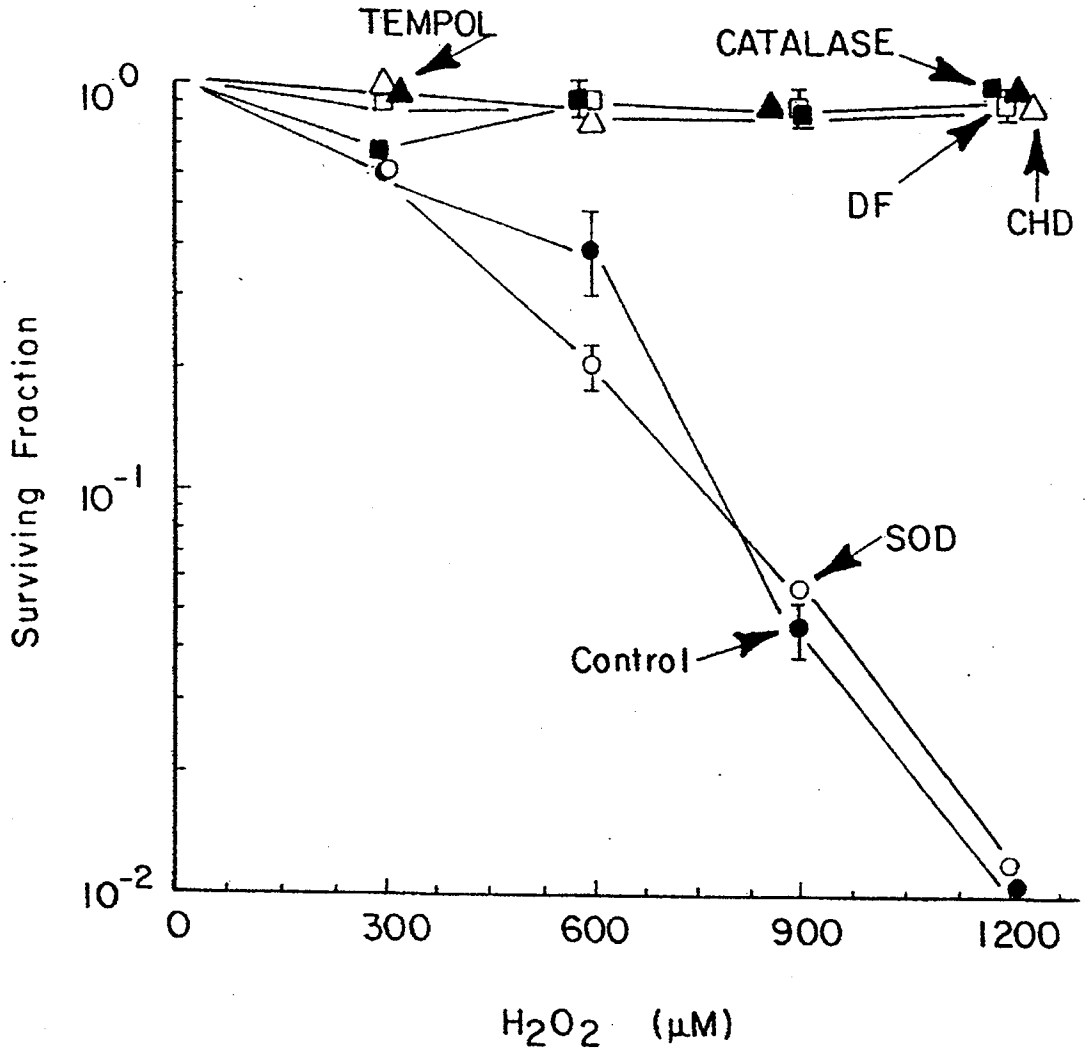

TEMPOL
CATALASE
DF
CHD
SOD
Control
10^0
10^{-1}
10^{-2}
Surviving Fraction
0
300
600
900
1200
H_2O_2 (μM)

FIG. 3

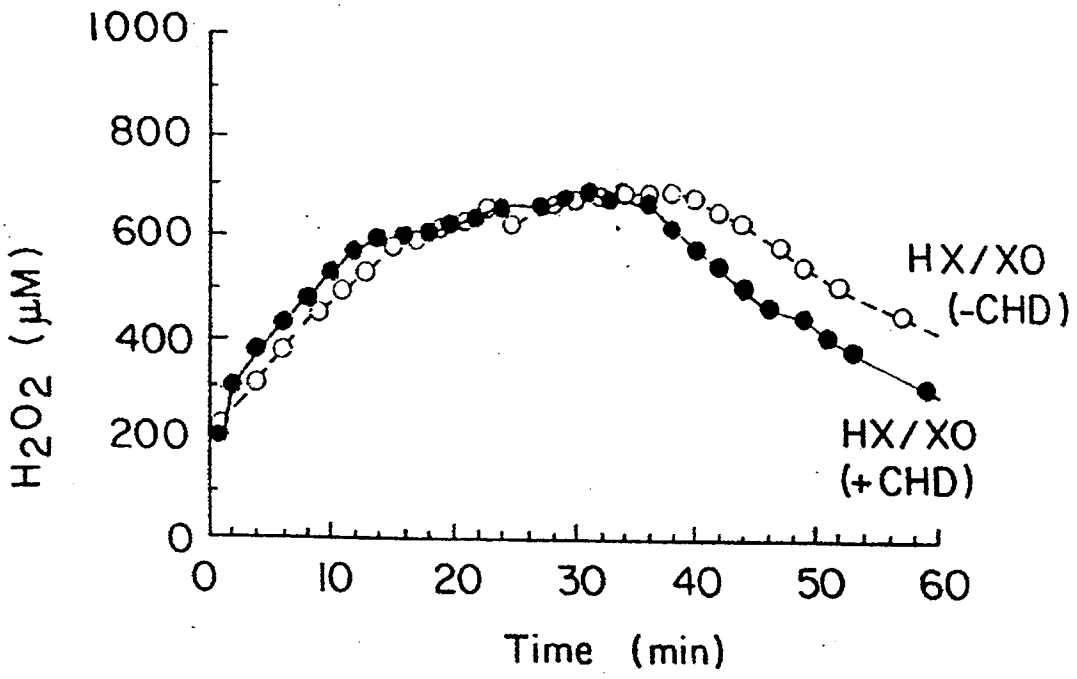

1000
800
600
400
200
0
H_2O_2 (μM)
0
10
20
30
40
50
60
Time (min)
HX/XO
(-CHD)
HX/XO
(+CHD)

FIG. 4

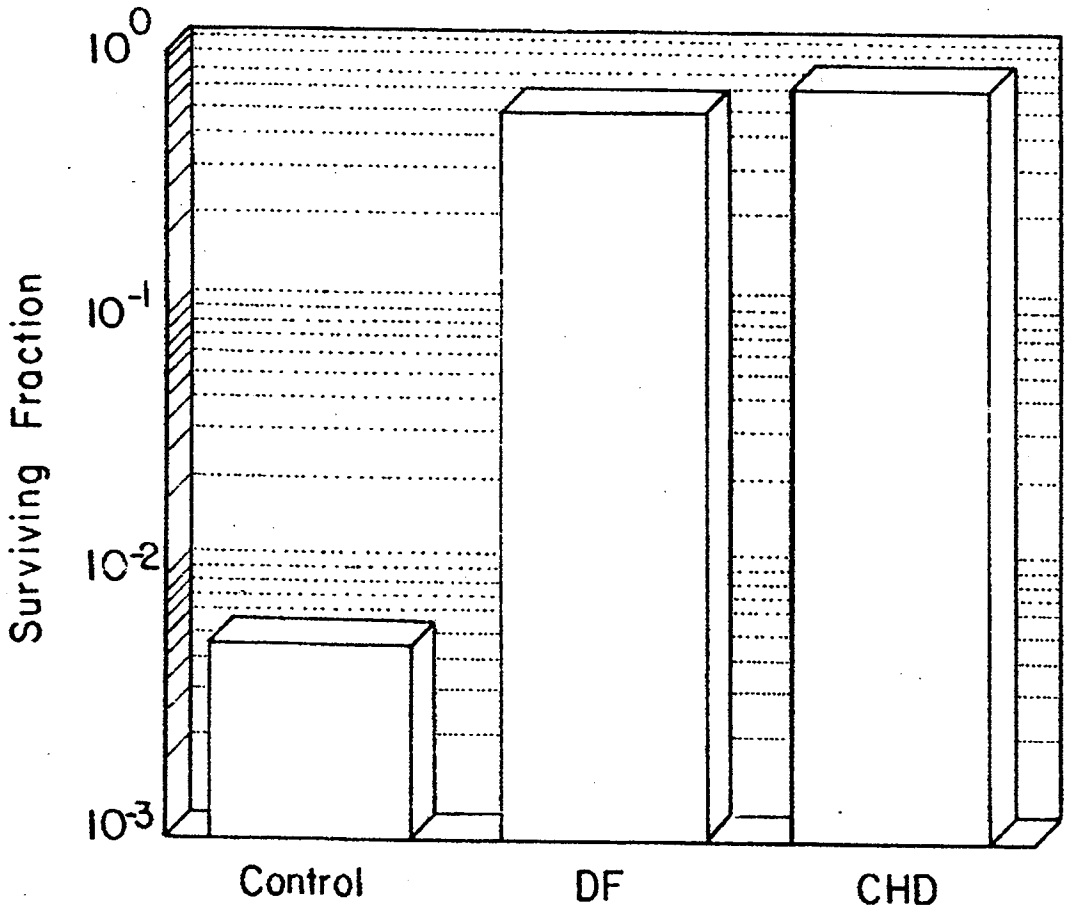


FIG. 5

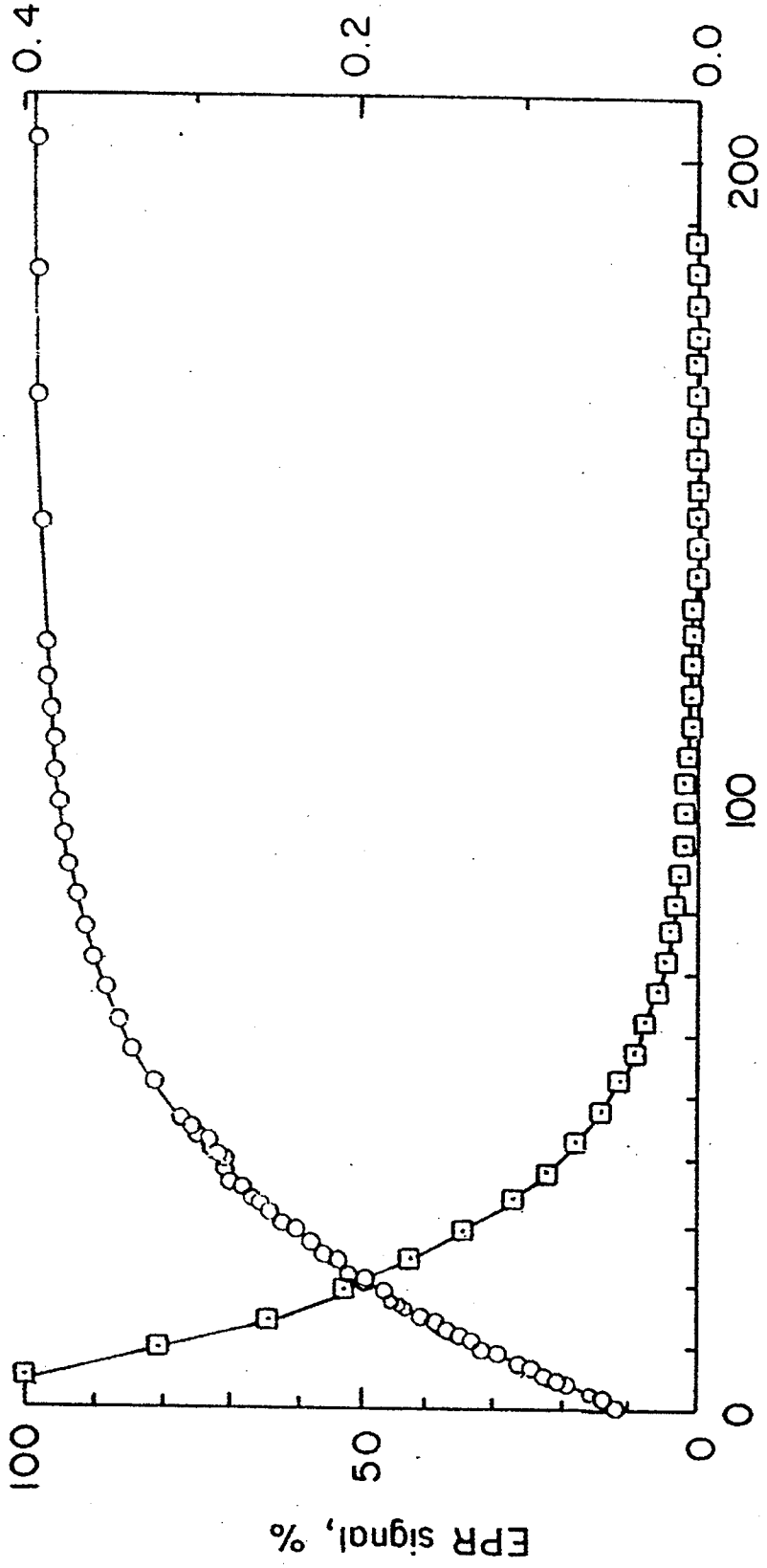


FIG. 6A

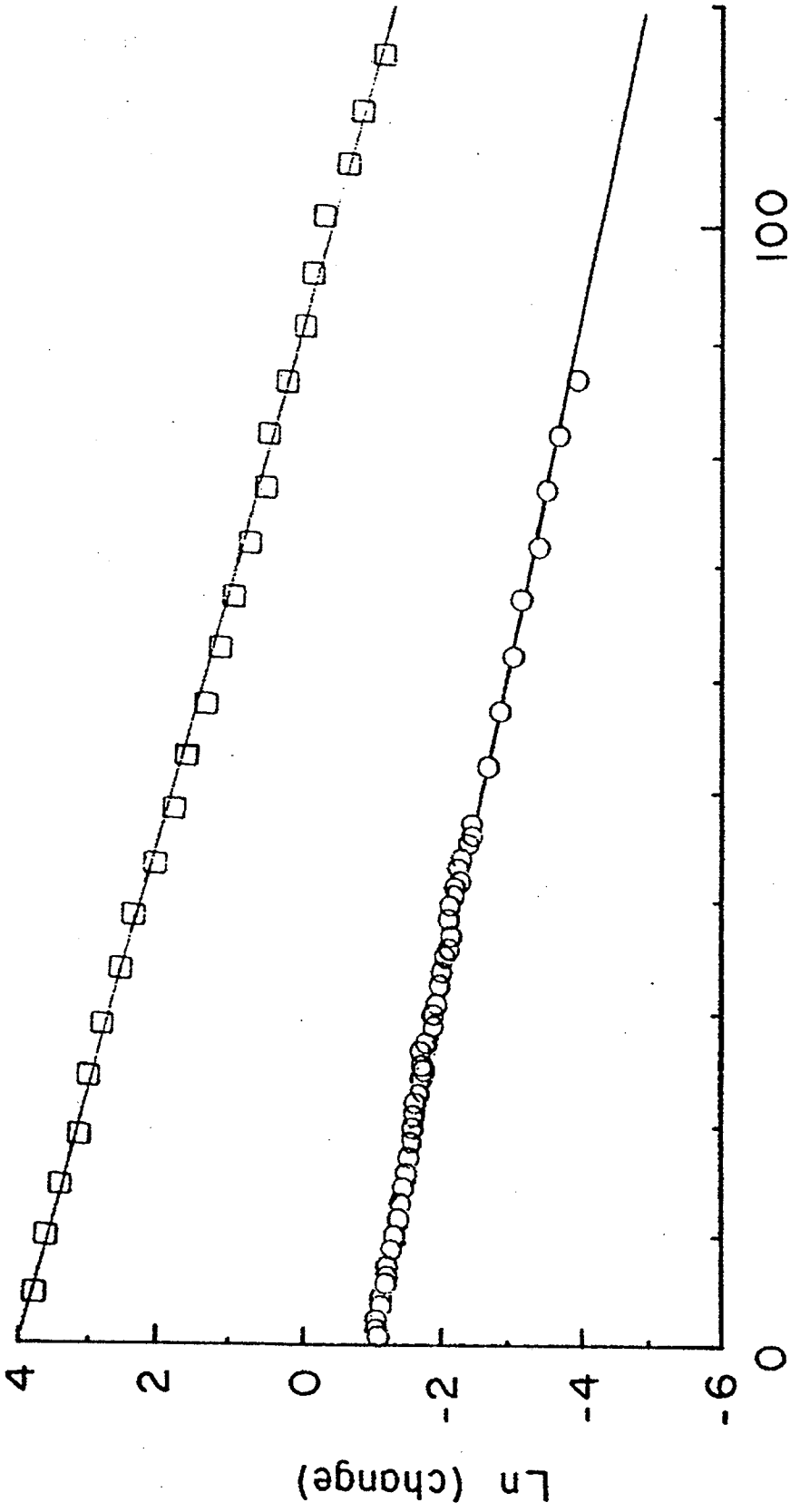


FIG. 6B

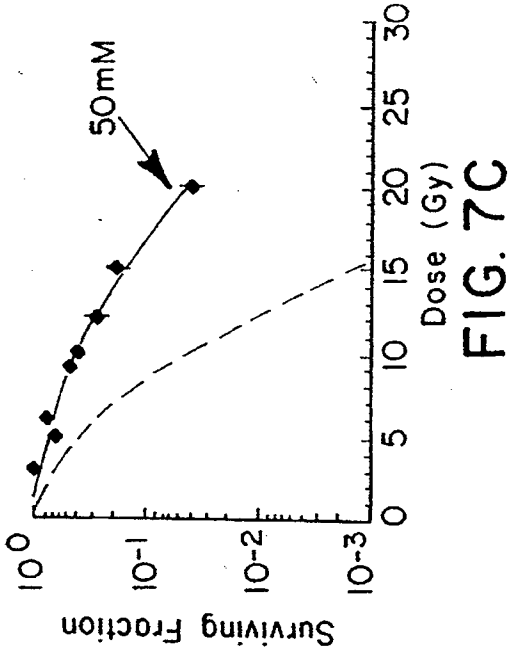


FIG. 7C

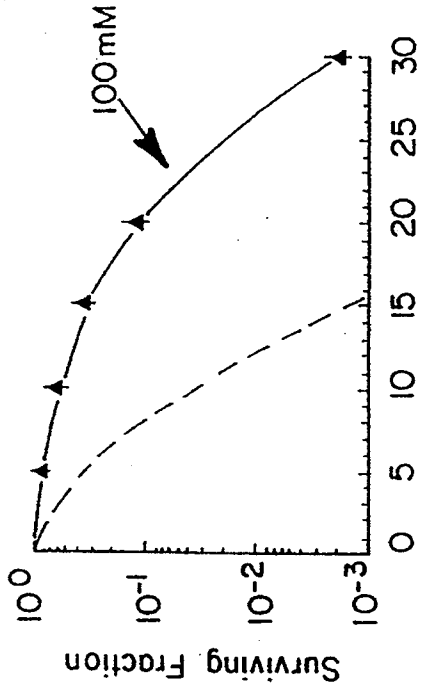


FIG. 7D

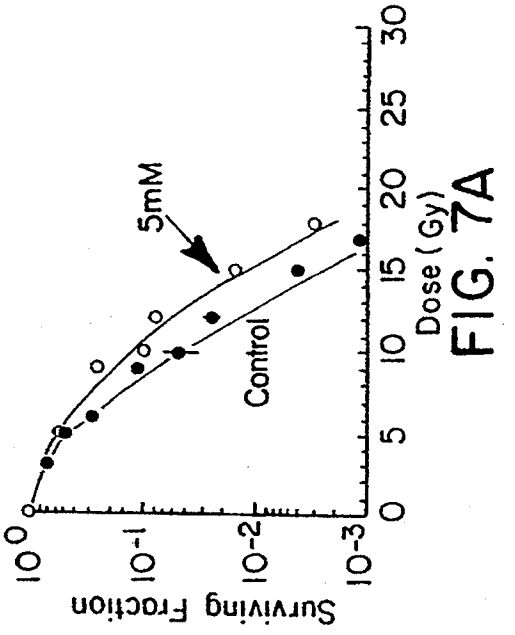


FIG. 7A

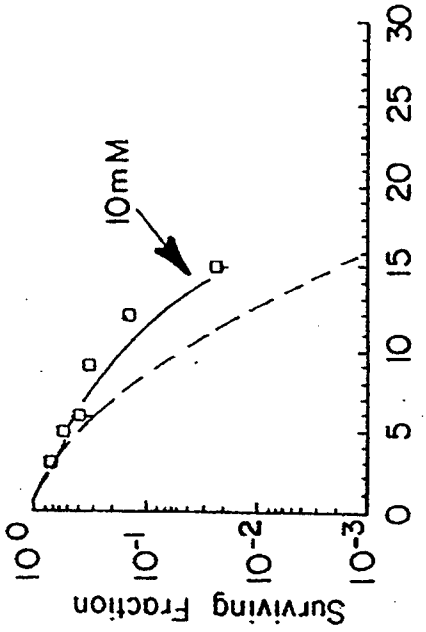


FIG. 7B

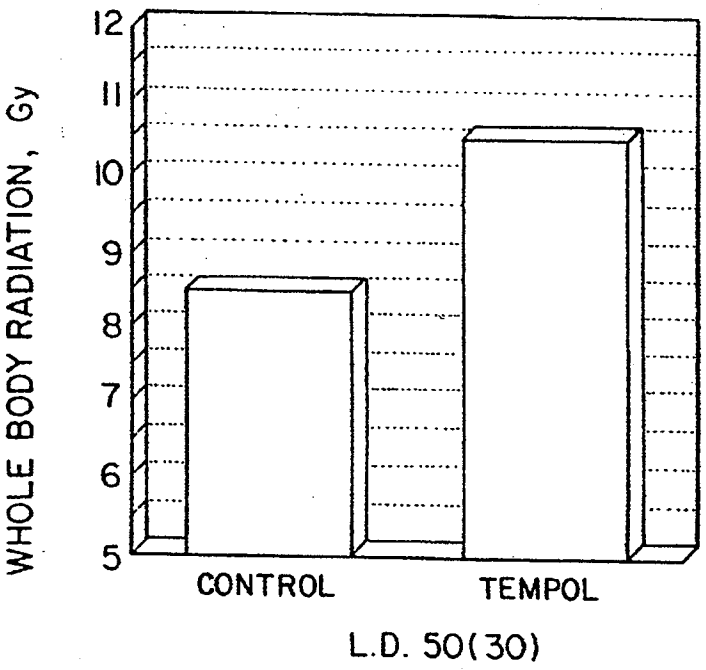

WHOLE BODY RADIATION, Gy
12
11
10
9
8
7
6
5
CONTROL
TEMPOL
L.D. 50(30)

FIG. 8

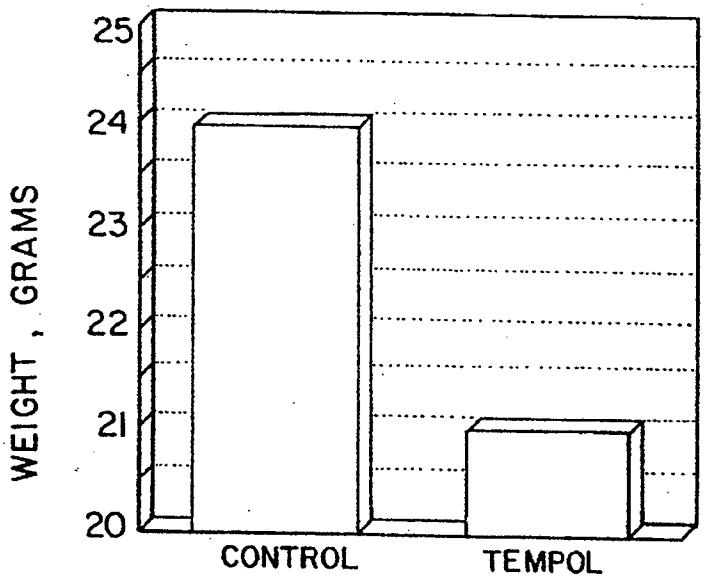

WEIGHT , GRAMS
25
24
23
22
21
20
CONTROL
TEMPOL

FIG. 9

NITROXIDES AS PROTECTORS AGAINST OXIDATIVE STRESS

This application is a continuation of application Ser. No. 07/494,532 filed on Mar. 16, 1990, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to pharmaceutical compositions containing nitroxide compounds useful in ameliorating the deleterious effects of toxic oxygen-related species in living organisms, and methods of using the same.

2. Description of Related Art

The utilization of oxygen by mammals carries both a blessing and a potential curse. The blessing is that all mammals require oxygen for life. The potential curse is that during the metabolism of oxygen, a variety of toxic oxygen-related species such as hydroxyl radical, (.OH); hydrogen peroxide, (H_2O_2); and superoxide, (O_2^-) are produced. Left unchecked, these free radical species could undoubtedly damage cells. However, cells have evolved elaborate detoxification and repair systems to rid themselves of these potentially toxic and undesirable metabolic by-products: superoxide dismutase (SOD) can convert superoxide to H_2O_2, and catalase (CAT) can convert H_2O_2 to H_2O.

Yet another means to detoxify H_2O_2 (and organoperoxides) is via the enzyme glutathione peroxidase (GPX), which with glutathione (GSH), also converts H_2O_2 to H_2O. Glutathione transferase (GST), in addition to its ability to conjugate and inactivate drugs and xenobiotics, also possesses peroxidase activity and can detoxify H_2O_2. These systems represent the major detoxification pathways for oxygen-derived free radicals species; however, there are doubtless other systems that may provide protection including protein sulfhydryls and other thiol-related enzymes that could be involved in repair mechanisms.

Despite the efficiency of these enzymatic systems, there is a small "leakage" of toxic species beyond the biochemical defense network. Of particular importance is the ultimate fate of H_2O_2 should it escape detoxification. H_2O_2, itself an oxidant capable of damaging biologically important molecules, can also undergo reduction via ferrous complexes to produce .OH. This reaction (often referred to as Fenton chemistry) produces the highly reactive .OH, which in the order of 10^{-9} seconds, can: 1) abstract electrons from organic molecules; 2) break chemical bonds; 3) initiate lipid peroxidation; and 4) react with another .OH to produce H_2O_2. It is not known whether chronic exposure to low level oxygen-derived free radical species is deleterious; however, it is postulated that the process of aging may be a manifestation of the organisms's inability to cope with sustained oxidative stress. Many modalities used in cancer treatment including x-rays and some chemotherapeutic drugs exert their cytotoxicity via production of oxygen-related free radicals, thereby imposing an added burden to normal detoxification systems. Additionally, free radicals and toxic oxygen-related species have been implicated in ischemia/reperfusion injury, and have long been thought to be important in neutrophil-mediated toxicity of foreign pathogens. Likewise, free radical damage has been implicated in carcinogenesis. The term "oxidative stress" has thus emerged to encompass a broad variety of stresses, some of which have obvious implications for health care.

There has been considerable interest in devising additional approaches, apart from inherent intracellular

detoxification systems, to protect cells, tissues, animals, and humans from the toxic effects of any agent or process that imposes oxidative stress. In the past few years, experimental studies have indicated that enzymes such as catalase and superoxide dismutase, and agents such as allopurinol and metal chelating compounds, afford protection against oxidative stress. None of these approaches is at present being applied to humans.

The application of the nitroxides of the instant invention is novel in this respect, and affords several unique advantages. Although the group of chemical compounds called stable nitroxide spin labels has had extensive biophysical use, they have never been used as antioxidants. They exhibit low reactivity with oxygen itself. Being low molecular weight, uncharged, and soluble in aqueous solution, they readily cross into the intracellular milieu. Enzymes such as catalase and superoxide dismutase do not. Therefore, the nitroxides should be superior to catalase and superoxide dismutase in that they can exert protection inside the cell. They are active within the biological pH range of about 5 to 8. Nitroxides are not proteins; therefore, the possibility of antigenic stimulation is remote. Previous low molecular weight superoxide dismutase mimics have all been metal dependent. The current agents do not contain metals, and problems with dissociation constants and deleterious metal induced reactions are therefore avoided. These compounds are apparently non-toxic at effective concentrations, and their lipophilicity can be controlled by the addition of various organic substituents, facilitating targeting of the molecules to specific organs or organelles where toxic oxygen-derived species are generated, to regions which are particularly susceptible to oxidative damage, or to the brain, if this is so desired. Previous radiation protectors have been sulfhydryl-group dependent. The current agents do not have a sulfhydryl group. Finally, previous use of these types of compounds as magnetic resonance contrast agents does not relate to their instant application as antioxidants.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to overcome the problems associated with the use of impermeable enzymatic detoxifying agents such as superoxide dismutase and catalase to protect living tissues from the deleterious effects of toxic products generated during oxygen metabolism. This is accomplished by providing a pharmaceutical composition containing the nitroxide compounds by any means, and methods for using the same as metal independent, low molecular weight antioxidant for use as:

1) Ionizing radiation protectants to protect skin, and to protect against mucositis, the effects of whole body radiation, and radiation-induced hair loss. Administration in these situations may be accomplished either via topical application as an ointment, lotion, or cream, intravenously or orally by pill or lozenge.

2) Protectants against increased oxygen exposures so as to avoid, for example, pulmonary adult respiratory distress syndrome (ARDS).

3) Protectants against oxygen-induced lenticular degeneration and hyaline membrane disease in infants, and against oxidative stress-induced cataracts. The compounds may also be used to protect against oxidative stress in patients undergoing oxygen therapy

or hyperbaric oxygen treatment. Administration under these circumstances may be accomplished via various routes including, for example, the use of eye drops, aerosol inhalation, or intravenous injection.

4) Reperfusion injury protectants effective in treating cardiovascular phenomena such as myocardial infarction and strokes, pancreatitis, or intestinal ulceration; to protect patients receiving organ transplants, and in organ preservation solutions.

5) Protectants for use in animal or plant cell culture media to prevent cytotoxicity due to excessive oxidation, for use in media designed for culturing aerobic microorganisms, for use in stabilizing labile chemical compounds which undergo spontaneous degradation by generating free radicals, for use in neutralizing free radicals which catalyze chain elongation during polymer formation, thereby terminating polymer elongation, and for use as a stabilizer for foods or food additives such as colors and flavors, especially in foods preserved via radiation treatment.

6) Biological antioxidants to protect humans and animals against agents such as the herbicide paraquat. In this circumstance, the pharmaceutical composition may be administered, for example, via inhalation as an aerosol to a subject exposed to paraquat. In addition, plants may be protected against such agents by, for example, spraying before or after exposure to such compounds.

7) Protectants against the cytotoxic effects of chemotherapeutic agents.

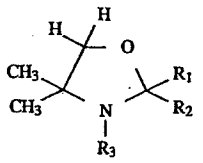
8) Protectants against mutagenic and carcinogenic agents. Administration in this situation or in 6, above, may be accomplished via oral ingestion, or parenterally.

9) Anti-inflammatory agents effective against arthritic conditions. For this purpose, the compositions may be administered parenterally, intra-articularly, or via oral ingestion.

10) Aging retardants. Administration for this purpose may be accomplished orally such as via a tablet supplement to the diet or parenterally.

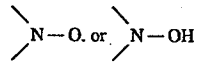
11) Oral or intravenous agents inducing weight reduction.

These and other objects are accomplished by providing a biologically compatible composition, comprising: an effective amount of a compound of the formula

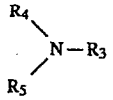


wherein R_1 is $-CH_3$; R_2 is $-C_2H_5$, $-C_3H_7$, $-C_4H_9$, $-C_5H_{11}$, $-C_6H_{13}$, $-CH_2-CH(CH_3)_2$, $-CHCH_3C_2H_5$, or $-(CH_2)_7-CH_3$, or wherein R_1 and R_2 together form spirocyclopentane, spirocyclohexane, spirocycloheptane, spirocyclooctane, 5-cholestane, or norbornane; R_3 is —O. or —OH, or a physiologically acceptable salt thereof which has antioxidant activity; and a biologically acceptable carrier.

Compounds which may be useful in the present invention also include any compound having a



group, or a salt thereof. These compounds can be represented broadly by the formula:



wherein R_3 is as defined above, and R_4 and R_5 combine together with the nitrogen to form a heterocyclic group. The atoms in the heterocyclic group (other than the N atom shown in the formula) may be all C atoms or may be C atoms as well as one or more N, O and/or S atoms. The heterocyclic group preferably has 5 or 6 total atoms. The heterocyclic group may be preferably a pyrrole, imidazole, oxazole, thiazole, pyrazole, 3-pyrroline, pyrrolidine, pyridine, pyrimidine, or purine, or derivatives thereof, for example.

Further compounds which may be useful in the present invention also include those wherein R_4 and R_5 themselves comprise a substituted or unsubstituted cyclic or heterocyclic groups.

Still further compounds which may be useful in the present invention also include oxazolidine compounds capable of forming an oxazolidine-1-oxyl.

Yet further compounds which may be useful in the present invention also include metal-independent nitroxides.

The present invention is also directed to methods for treating the deleterious effects of harmful oxygen-derived metabolic products, as listed above.

Physiologically acceptable salts include acid addition salts formed with organic and inorganic acids, for example, hydrochlorides, hydrobromides, sulphates such as creatine sulphate salts, phosphates, citrates, fumarates and maleates. The compounds of the invention have been shown to exhibit little or no in vitro cytotoxicity at concentrations of from 1–5 mM for 5 hours.

The compounds of the present invention can be used for the treatment of the toxic effects of oxidative stress in a variety of materials, cells and mammals including humans, domestic and farm animals, and laboratory animals such as hamsters, mice, rats, monkeys, etc. It is contemplated that the invention compounds will be formulated into pharmaceutical compositions comprising an effective antioxidant amount of the compounds of formula (I) and pharmaceutically acceptable carriers. An effective antioxidant amount of the pharmaceutical composition will be administered to the subject or organism, human, animal, or plant, in a manner which prevents the manifestations of oxidative stress. The amount of the compound (I) and the specific pharmaceutically acceptable carrier will vary depending upon the host and its condition, the mode of administration, and the type of oxidative stress condition being treated.

In a particular aspect, the pharmaceutical composition comprises a compound of formula (I) in effective unit dosage form. As used herein the term "effective unit dosage" or "effective unit dose" is denoted to mean a predetermined antioxidant amount sufficient to be effective against oxidative stress in vitro or in vivo. Pharmaceutically acceptable carriers are materials useful for the purpose of administering the medicament, which are preferably non-

toxic, and may be solid, liquid or gaseous materials which are otherwise inert and medically acceptable, and are compatible with the active ingredients. The pharmaceutical compositions may contain other active ingredients such as antimicrobial agents and other agents such as preservatives.

These pharmaceutical compositions may take the form of a solution, emulsion, suspension, ointment, cream, aerosol, granule, powder, drops, spray, tablet, capsule, sachet, lozenge, ampoule, pessary, or suppository. They may be administered parenterally, intramuscularly or subcutaneously, intravenously, intra-articularly, transdermally, orally, buccally, as a suppository or pessary, topically, as an aerosol spray, or drops.

The compositions may contain the compound in an amount of from 0.1%–99% by weight of the total composition, preferably 1 to 90% by weight of the total composition. For intravenous injection, the dose may be about 0.1 to about 300 mg/kg/day. If applied topically as a liquid, ointment, or cream, the compound may be present in an amount of about 0.01 to about 100 mg/ml of the composition. For inhalation, about 0.1 to about 200 mg/kg body weight of the compound should be administered per day. For oral administration, the compound should be administered in an amount of about 0.1 to about 300 mg/kg/day.

The invention also provides a method for treating the effects of oxidative stress due to the production of harmful oxygen-derived species which comprises administering an effective antioxidant amount of the above-mentioned compound to a organism, material, mammal or human susceptible to oxidative stress. Such stress includes that due to oxidizing agents, increased oxygen exposure, oxygen-induced degeneration or disease, reperfusion injury, ionizing radiation, carcinogenic, chemotherapeutic, or mutagenic agents, aging, or arthritis.

Reperfusion injury may include myocardial infarction, strokes, pancreatitis, and intestinal ulceration, while oxidative stress due to increased oxygen exposure includes pulmonary adult respiratory distress syndrome. Other oxidative stresses amenable to treatment with the compounds of the instant invention include oxygen-induced lenticular degeneration, cataracts or hyaline membrane disease in infants, or oxidative stress occurring during oxygen therapy or hyperbaric oxygen treatment.

Finally, in a further aspect of the invention, the compound of the instant invention can be used to prolong the storage life of human or animal cells, tissues, or organs by contacting these materials with a storage solution containing an effective amount of such compound, or to induce weight reduction in humans or animals.

The terms "biologically compatible" refer to a composition which does not cuase any adverse effects to an organism to which it is applied. The composition is preferably free of toxic substances or other substances which would render it unsuitable for the intended use.

The term "parenteral" includes an administration by injection such as intravenous, intramuscular, or subcutaneous.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows EPR spectra of CHD and TEMPO demonstrating the partitioning of each nitroxide (1 mM) in both the intra- and extra-cellular space of V79 cells. The EPR signal intensity of the total concentration of CHD (intra- and extra-cellular) in 6.4×10^7 V79 cells/ml traces (A and C) and in the presence of 110 mM trioxalatochrmiate (CrOx) (traces B and D). The gains for individual spectra are as cited in the individual figures.

FIG. **2** shows a survival curve for Chinese hamster V79 cells exposed to HX/XO (hypoxanthine/xanthine oxidase) in the presence of various additives, including the nitroxides CHD and TEMPOL, which fully protected the cells. Chinese hamster V79 cells in full medium at 37° C. were exposed to 0.05 U/ml XO+0.5 mM HX for various time periods in the presence of various additives: (●), control, no additives; (■), 100 U/ml catalase; (○) , 100 U/ml SOD; (□), 500 μM DF (desferrioxamine), preincubated for 2 h with the cells prior to addition of XO; (Δ), 5 mM CHD; (▲), 5 mM TEMPOL.

FIG. **3** shows a survival curve for cells exposed to H_2O_2 in the presence of various additives, including the nitroxides CHD and TEMPOL, which fully protected the cells. The effect of various agents on cell survival was measured by clonogenic assay of Chinese hamster V79 cells exposed in full medium at 37° C. to various concentrations of H_2O_2 for 1 h; (●), control, no additives; (■), 100 U/ml catalase; (○), 100 μg/ml SOD; (□), 500 μM DF, preincubated 2 h with the cells prior to H_2O_2 addition; (Δ), 5 mM CHD; (▲), 5 mM TEMPOL.

FIG. **4** shows the effect of nitroxide on the accumulation and decay of H_2O_2 in a tissue culture of cells exposed to HX/HO. Chinese hamster V79 cells were plated in full medium and incubated at 37° C. with 5 mM HX+0.04 U/ml XO, sampled at various time points, and assayed for H_2O_2 using a hydrogen peroxide selective electrode.

FIG. **5** shows the survival of Chinese hamster V79 cells exposed to 600 μM H_2O_2±DF or CHD in full medium at 37° C. for 1 h under hypoxic conditions.

FIG. **6** shows the reaction between CHD and DNA-Fe(II): CHD in 50 mM MOPS buffer pH 7.0 was anoxically mixed at 22° C. with DNA-Fe(II). All solutions always contained 0.1 mg/ml Salmon DNA. The appearance of DNA-Fe (III) was spectrophotometrically monitored at 353 nm, whereas the spin-loss of CHD was monitored by following its EPR signal. To study the time-dependence of ΔOD_{353nm} (○), 1 mM CHD was mixed with 0.1 mM Fe(II). To follow the spin-loss of CHD (□), 1 mM Fe(II) was mixed with 0.1 mM CHD. Inset: Time-dependence of ln{EPR signal} (□); and of $\ln\{(OD_{\infty}-OD_t\}$ (○).

FIG. **7** shows the clonogenic survival of Chinese hamster V79 cells treated with varying concentrations of TEMPOL 10 min prior to radiation.

FIG. **8** shows the protection afforded whole animals treated with TEMPOL prior to whole body irradiation. Six week old female C3H mice were given 275 mg/kg TEMPOL intraperitoneally 10 mins prior to irradiation with 3 Gy to 13 Gy. Controls were given saline.

FIG. **9** shows the average weights of control and TEMPOL treated mice.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

EXAMPLE 1

Synthesis and SOD-like activity of oxazolidine derivatives in vitro.

Desferrioxamine (DF) was a gift from Ciba Geigy; hypoxanthine (HX) was purchased from Calbiochem-Boehringer Co.; 2,2,6,6 -tetramethylpiperidine-1-oxyl (TEMPO), 4-hydroxy-2,2,6,6 -tetramethylpiperidine-1-oxyl

(TEMPOL), 4-hydroxypyrazolo[3,4, -d]-pyrimidine (allopurinol), p-toluene sulfonic acid, 2-amino-2 -methyl-1-propanol, 2-butanone, and cyclohexanone were purchased from Aldrich Chemical Co.; trioxalato chromiate(III) (CrOx) was purchased from Pfaltz and Bauer, Inc., and recrystallized; xanthine oxidase (EC 1.2.3.2. xanthine: oxygen oxidoreductase) grade III from buttermilk, superoxide dismutase (SOD), and grade V ferricytochrome c were obtained from Sigma. H_2O_2 was bought from Fisher Scientific Co. XO was further purified on a G25 sephadex column. All other chemicals were prepared and used without further purification. Distilled-deionized water was used throughout all experiments.

CHD, 2-spirocyclohexane doxyl (2-spirocyclohexane-5,5 -dimethyl-3-oxazolidinoxyl) and OXANO, 2-ethyl-2,5,5-trimethyl-3 -oxazolidine-1-oxyl as well as other nitroxides were synthesized as described by Keana et al (J. Am. Chem. Soc., 89, 3055–3056, 1967). For the general synthesis of the cyclic amines, the appropriate starting ketone was reacted with 2-amino-2-methyl-1-propanol in benzene in the presence of catalytic amounts of p-toluene sulfonic acid. As the cyclic structure formed, water was eliminated. The volume of water collected in a Dean Stark apparatus was monitored and used to gauge the reaction progress. The amines thus produced were purified through fractional distillation under reduced pressure, characterized by 220 MHz ^1H NMR, IR, UV, either EI or CI mass spectroscopy, and subsequently oxidized to the corresponding nitroxides using m-chloroperbenzoic acid. The nitroxides were purified by silica flash chromatography (Still et al, J. Org. Chem., 43, 2923–2925, 1978). Water/octanol ratios were determined by placing a quantity of nitroxide in water+octanol within a separatory funnel. The mixture was shaken thoroughly and allowed to separate for 15 min, whereupon aliquots were taken from both fractions and the ratio of nitroxide distribution was determined using electron paramagnetic resonance (EPR) spectroscopy, by comparing the intensities of signal obtained under N_2.

To check whether oxazolidinoxyl derivatives other than OXANO manifest SOD-like activity, several nitroxides having different ring substituents were synthesized. Table 1A shows representative synthesized nitroxides with accompanying physical characteristics.

TABLE 1A

	Five-Membered Oxazolidine-1-oxyl (Dozyl)				
	Nitroxide	Ring substituents		Yield	Partition[#]
	notation	R_1	R_2	(%)	coefficient
I	OXANO	CH_3	C_2H_5	42	10
II		CH_3	C_5H_{11}	52	145
III		CH_3	C_4H_9	49	58
IV	CHD	spirocyclohexyl		77	80
V		CH_3	C_6H_5	22	720

[#]Octanol:water

Exposure of these 5-membered cyclic nitroxides to O_2^- flux formed by HX/XO resulted in a decrease in their EPR signal, as previously found for OXANO (Samuni et al, Free Rad. Biol. Med., 6, 141–148, 1989). For EPR experiments, samples (0.05–0.1 ml) either of solutions of chemicals or cell suspensions were drawn by a syringe into a gas-permeable teflon capillary of 0.8 mm inner diameter, 0.05 mm wall thickness (Zeus Industrial Products, Inc. Raritan, N.J.). Each capillary was folded twice, inserted into a narrow quartz tube which was open at both ends (2.5 mm ID), and then placed horizontally into the EPR cavity. During the experiments, gases of desired compositions were blown around the sample without having to disturb the alignment of the tube within the EPR cavity. EPR spectra were recorded in a Varian E4 (or E9) X-band spectrometer, with field set at 3357G, modulation frequency of 100 KHz, modulation amplitude of 1G and non-saturating microwave power. The EPR spectrometer was interfaced to an IBM-PC through an analog-to-digital converter and a data translation hardware (DT2801) and the spectra were digitized using commercial acquisition software, enabling subtraction of background signals. To study the kinetics of the spin-loss, the spectra were deliberately overmodulated, and the magnetic field was kept constant while the intensity of the EPR signal was followed.

After terminating the HX/XO reaction by allopurinol, the nitroxide spin-loss was reversed by adding 0.5 mM ferricyanide, indicating that O_2^- reduces the nitroxide to its respective hydroxylamine (Samuni et al, Free Rad. Biol. Med., 6, 141–148, 1989). On the other hand, no effect of O_2^- on the EPR signal of 6-membered ring nitroxides such as TEMPO and TEMPOL was detectable (see Table 1B) .

TABLE 1B

Kinetic Data: SOD-like Activity of 5- and 6-Membered Cyclic Nitroxides Chemical Structure				
Nitroxide notation	TEMPOL	TEMPO	OXANO	CHD
Steady state EPR signal (%)[a]	100	100	50	30
$kR'RNO + O_{1/2}$ ($M^{-1}s^{-1}$)[b]	3.4×10^5	1.3×10^6	1.1×10^3	3.5×10^3

[a]Steady-state EPR signal of nitroxides (% from total R'RNO + R'RNOH) after exposure to 5 mM HX + 0.03 U/ml XO in air-saturated PBS pH 7.2.
[b]Rate constants were determined at low ionic strength (10 mM HEPES), pH 7.0, and 22° C.

The failure of superoxide to affect TEMPO and TEMPOL apparently suggested that 6-membered cyclic nitroxides lack SOD-like activity. As a further check, the reaction of representatives of both 5- and 6-membered cyclic nitroxides with O_2^- was studied. The SOD-inhibitable ferricytochrome c reduction assay (Fridovich, *Handbook of Methods for Oxygen Radical Research*, 213–215, 1985) was used to determine rate constants of reaction with O_2^-. Superoxide radicals were generated at 25° C. in aerated phosphate buffer (50 mM) containing 50 μM DTPA, 5 mM HX, and 10–50 μM ferricytochrome c (with or without 65 U/ml catalase). The reaction was started by adding 0.01 U/ml XO and the rate of ferricytochrome c reduction, in the absence (V) and in the presence (v) of various nitroxides, was spectrophotometrically followed at 550 nm. Both reference and sample cuvettes contained all the reagents, with the reference cuvette containing 100 units/ml SOD, thereby eliminating spurious reactions from interfering with the determination of rate constants. Data were analyzed by plotting V/v as a function of [nitroxide] and k_1 was calculated knowing $k_{CytC+superoxide}$, according to: $(V/v)-1= k_1 x[\text{nitroxide}]/k_{CytC+superoxide} X[\text{Cyt-}c^{III}]$.

Via this assay, all the nitroxides listed below have been shown to function as superoxide dismutase mimics.

TABLE 2

Physical data of 2-substituted-5,5-dimethyl-3-oxazolidines. Generalized Structure, R groups shown in table.

Oxazolidine (A–I)	R1	R2	Yield %	b.p. C (760 mmHg)
A	CH3	C2H5	42	162–165
C	CH3	C3H7	36	169–170
D	CH3	C4H9	71	185–188
E	CH3	C5H11	52	204–205
F	CH3	C6H13	54	225–230
G	CH3	CH2CH(CH3)2	49	168–170
H	CH3	CHCH3C2H5	25	184–185
I	CH3	(CH2)7CH3	57	165–166

Oxazolidine (J–R)	Alicyclic substituent	Yield %	b.p. C (760 mmHg)
J	spirocyclopentane	61	133–135 (75)
K	spirocyclohexane	77	208–211 (760)
L	spirocycloheptane	50	145–147 (35)
M	spirocyclooctane	40	234–235 (760)
N	5-cholestane	—	—
O	norbornane	53	178–180 (80)

The rate constants of the synthetic nitroxides' reaction with O_2 at low ionic strength (10 mMHEPES) and pH 7.0 ranged from 1.1×10^3 to $1.3\times10^6 M^{-1}s^{-1}$, as compared with $2.3\times10^9 M^{-1}s^{-1}$ for k_{cat} of native SOD.

None of the nitroxides shown (in the last two tables) exhibited cytotoxicity determined by clonogenic assay in V79 cells exposed for 1 h at 5 mM. For subsequent studies, the most lipophilic nitroxide, CHD, and the most hydrophilic one, TEMPOL, were chosen.

EXAMPLE 2

Nitroxide Intracellular Localization.

FIG. 1A and C illustrate the EPR signal from 1 mM CHD and TEMPOL, respectively, suspended with 6.4×10^7 V79 cells/ml. This EPR signal represents the total concentration of intra- and extra-cellular CHD. Trioxalato-chromiate is a paramagnetic broadening agent which remains excluded from the intracellular volume space and causes the EPR signal from extracellular species to become non-detectable (Lai, Biophys. J., 52, 625–628, 1987). When cells were added to CHD or TEMPOL in the presence of 110 mM trioxalatochrmiate, a much smaller yet observable intracellular signal was detected as shown in FIG. 1B and D. The observable line broadening and loss of the hyperfine structure of the intracellular signal indicate that CHD, though not the TEMPOL, has decreased freedom of motion (anisotropy) within the intracellular environment and is located primarily in a membranous compartment as can be anticipated based on the difference between their lipophilicities.

EXAMPLE 3

Protection of Cells Against Oxidative Damage.

Chinese hamster V79 cells were grown in F12 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Survival was assessed in all studies by the clonogenic assay. The plating efficiency range between 80–90%. Stock cultures of exponentially growing cells were trypsinized, rinsed, and plated (5×10^5 cells/dish) into a number of 100 mm petri dishes and incubated 16h at 37° C. prior to experimental protocols. Cells were exposed for lh at 37° C. to either 0.5 mM hypoxanthine (HX)+0.05 U/ml of xanthine oxidase (XO) for varying lengths of time, or to H_2O_2 at different concentrations. To assess possible modulation in cytotoxicity, catalase, 100 U/ml; SOD, 100 μg/ml; DF, 500 μM; and 5 mM of each of the nitroxides from Table 1 were added to parallel cultures. CHD was prepared in a stock solution in ethanol and diluted into medium such that the final concentration was 5 mM. This resulted in a final concentration of 1% ethanol in the medium which was not cytotoxic and did not influence the cellular response to HX/XO or H_2O_2. TEMPOL is water soluble and was prepared directly in tissue culture medium. Neither catalase, SOD, DF, CHD, nor TEMPOL were cytotoxic alone in the concentrations used. DF was added either 2 h prior to or during treatment while the other agents were present only during HX/XO or H_2O_2 treatment. Following treatment, cells were rinsed, trypsinized, counted, and plated for macroscopic colony formation. For each dose determination cells were plated in triplicate and the experiments were repeated a minimum of two times. Plates were incubated 7 days, after which colonies were fixed with methanol/acetic acid, stained with crystal violet, and counted. Colonies containing >50 cells were scored. Error bars represent S.D. of the mean and are shown when larger than the symbol.

Some studies required exposure to H_2O_2 under hypoxic conditions. For these studies, cells dispersed in 1.8 ml of medium were plated into specially designed glass flasks (Russo et al, Radiat. Res., 103, 232–239, 1985). The flasks were sealed with soft rubber stoppers, and 19-gauge needles were pushed through to act as entrance and exit ports for a humidified gas mixture of 95% nitrogen/5% CO_2 (Matheson Gas Products). Each flask was also equipped with a ground glass side arm vessel which when rotated and inverted could deliver 0.2 ml of medium containing H_2O_2 at a concentration which when added to the cell monolayer resulted in final concentration of H_2O_2 of 600 μM. Stoppered flasks were connected in series and mounted on a reciprocating platform and gassed at 37° C. for 45 min. This gassing procedure results in an equilibrium between the gas and liquid phase (in both the medium over the cell monolayer and in the solution in the sidearm) and yielded oxygen concentrations in the effluent gas phase of <10 ppm as measured by a Thermox probe (Russo et al, Radiat. Res., 103 232–239, 1985). After 45 min of gassing, the hypoxic $H2O_2$ solution was added to the cell monolayer culture. The cells were exposed to H_2O_2 for 1 h under hypoxic conditions. N_2 gas flow was maintained during the H_2O_2 exposure. In parallel flasks, DF and CHD were added as described above, and were present during the entire gassing procedure. Following treatment, cell survival was assessed as described above.

Hydrogen peroxide was assayed using a YSI Model 27 Industrial Analyzer (Yellow Springs Instruments) equipped with a selective electrode for H_2O_2. For analysis of cellular preparations, the cells, except during the brief time required for removal of aliquots for analysis, were kept in T25 culture flasks maintained at 37° C. in complete medium (pH 7.2). Aliquots of 25 μl were sampled from the reaction or cell preparation system at varying time points and injected into the analyzer. $[H_2O_2]$ was determined after calibrating the instrument with known concentrations of H_2O_2. The concentrations of standard H_2O_2 solutions were calibrated using iodometric assay (Hochanadel, J. Phys. Chem., 56, 587–594, 1952).

To expose the cells to oxidative stress they were incubated with HX/XO. FIG. 2 shows a survival curve for cells exposed to HX/XO. Cell survival was not altered when SOD was present during the HX/XO exposure. In contrast, 5 mM

CHD or TEMPOL fully protected the cells. The other nitroxides, presented in Table I, afforded similar protection (data not shown). FIG. 2 also shows that either catalase or DF provides complete protection from HX/XO-derived damage. Complete protection by DF required a 2 h preincubation with DF before cells were exposed to HX/XO, whereas DF addition simultaneously with HX/XO offered only partial protection (data not shown).

One interpretation of the data shown in FIG. 2 is that H_2O_2 is the principal cytotoxic species produced by the HX/XO system (Link & Riley, Biochem. J., 249, 391–399, 1988). This assumption is based on the fact that extracellular catalase provided complete protection from HX/XO (FIG. 2). To test if cell protection by the SOD-mimic resulted from detoxifying H_2O_2, cells were exposed to H_2O_2 as shown in FIG. 3. The results of these experiments were identical to those shown in FIG. 2, in that SOD did not protect, but catalase, DF, TEMPOL, and CHD provided complete protection against H_2O_2 cytotoxicity. At this point it was questioned if CHD might have other features apart from acting as a SOD mimic, namely, whether CHD affects H_2O_2 concentration. FIG. 4 shows the concentration of H_2O_2 in tissue culture exposed to HX/XO. With time there was a build-up followed by a slow decline in $[H_2O_2]$. The presence of CHD did not significantly alter the pattern of H_2O_2 generation by HX/XO. Thus, the cellular protection afforded by CHD to HX/XO and H_2O_2 could not be attributed to a direct reaction of CHD with HO_2.

Even with direct exposure of cells to H_2O_2, there is the possibility that superoxide could be produced intracellularly as a result of the H_2O_2 a treatment. If superoxide were produced intracellularly, CHD protection of cells from HX/XO and H_2O_2 might be expected, given the findings that CHD can penetrate intracellular spaces as shown in FIG. 1. To test if the cytoprotection provided by CHD was solely a result of its reaction with superoxide, CHD effectiveness was examined when H_2O_2 was applied to cells incubated in a hypoxic environment, conditions in which the chance for superoxide formation would be significantly limited. As is seen in FIG. 5, CHD protects against H_2O_2 cytotoxicity even under hypoxic conditions.

FIG. 5 also shows that DF provides complete protection to H_2O_2 cytotoxicity under hypoxic conditions. The pattern of DF protection shown in FIGS. 2, 3, and 5 suggested that the cytotoxicity of HX/XO and H_2O_2 may directly involve intracellular reduction of H_2O_2 by ferrous ion to produce the highly toxic .OH. It was also questioned whether the aerobic and hypoxic protection by CHD to H_2O_2 exposure was a result of CHD directly accepting electrons from ferrous ions, thereby preventing generation of .OH. Because cellular iron is chelated, the possible reaction of nitroxide with chelated iron(II) was examined by repeating the experiment in the presence of DNA. To study the possibility of nitroxide-induced oxidation of transition metals, CHD was hypoxically mixed with iron(II) in the presence of 0.1 mg/ml Salmon DNA. Consequently, DNA-Fe(III) was formed and the nitroxide EPR signal disappeared. The reaction kinetics were investigated by maintaining either CHD or Fe(II) in excess while the absorbance due to DNA-Fe(III) and the nitroxide spin-loss were monitored respectively (FIG. 6). Both the decay of the EPR signal and the appearance of the OD_{353nm} obeyed pseudo 1^{st} order kinetics from which the 2^{nd} order reaction rate constant was calculated as 44 $M^{-1}s^{-1}$ or 33 $M^{-1}s^{-1}$ using the data from EPR or optical absorption, respectively. When TEMPOL was hypoxically mixed with DNA-Fe(II), a similar reaction took place having a 2^{nd} order reaction rate constant of 40 $M^{-1}s^{-1}$. The spin-loss was completely reversed by adding 2 mM ferricyanide, thus indicating that DNA-Fe(II) reduced the respective nitroxide to its hydroxylamine.

EXAMPLE 4

In Vitro and In Vivo Protection Against Ionizing Radiation by Nitroxide.

Chinese hamster V79 cells were treated with varying concentrations of TEMPOL 10 min prior to irradiation. The clonogenic survival compared to control cells is shown in FIG. 7. The extent of protection for 100 mM TEMPOL was approximately 2.5 fold.

The protection afforded whole animals by TEMPOL was evaluated in six week, old female C3H mice given 275 mg/kg TEMPOL intraperitoneally 10 min prior to whole body radiation doses ranging from 3 Gy to 13 Gy. Controls were given saline. Survival was recorded 30 days after exposure to radiation. The $LD_{50(30)}$ refers to that dose of radiation at which 50% of the mice survived 30 days after exposure. As can be seen in FIG. 8, mice treated with TEMPOL had an approximately 25% higher $LD_{50(30)}$, demonstrating protection from whole body radiation and no toxicity.

The above results demonstrate that TEMPOL provides radio-protection at both the in vitro and in vivo level.

EXAMPLE 5

Nitroxide-Induced Weight Loss in Animals.

Six week old female C3H mice (6 animals in each group) were allowed to drink an unlimited supply of water alone (control) or 4 -hydroxy tempo (TEMPOL) dissolved in water at a concentration of 10 mg per ml. Chronic oral administration (>3 weeks) results in no apparent toxicity to the animals, but a reduction of weight compared to controls by 12.5% as shown in FIG. 9. Thus, nitroxide given over extended periods appears to cause weight loss in animals.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

What is claimed is:

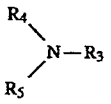
1. A pharmaceutical composition, comprising an anti-oxidative stress effective amount of a compound selected from the group consisting of a metal-independent nitroxide, an oxazolidine compound capable of forming an oxazolidine-1-oxyl, and a physiologically acceptable salt of either of the foregoing, and

a biologically acceptable carrier,

wherein said pharmaceutical composition is in a form selected from the group consisting of an emulsion, suspension, ointment, cream, aerosol, granule, powder, spray, tablet, capsule, sachet, lozenge, ampoule, pessary, and suppository, and

wherein said effective amount of said compound is sufficient to protect biological material from oxidative stress.

2. The pharmaceutical composition of claim 1, wherein the compound has the formula:



wherein R_3 is selected from the group consisting of —O. and —OH, and R_4 and R_5 combine together with the nitrogen to form a heterocyclic group, or

wherein R_4 and R_5 themselves comprise a substituted or unsubstituted cyclic or heterocyclic group,

or a physiologically acceptable salt thereof.

3. The pharmaceutical composition of claim 1, wherein the compound is present in an amount of from to 1 to 90% by weight of the total composition.

4. The pharmaceutical composition of claim 1, wherein said physiologically acceptable salt is selected from the group consisting of a hydrochloride, hydrobromide, sulphate, phosphate, citrate, fumarate, maleate and mixtures thereof.

5. The pharmaceutical composition of claim 1, wherein said biologically acceptable carrier is a non-toxic gas.

6. The pharmaceutical composition of claim 1, further comprising an antimicrobial agent and/or a preservative.

7. The pharmaceutical composition of claim 1, in a form capable of being administered, transdermally, orally, bucally, or as a suppository, a pessary, an aerosol, or drops.

8. The pharmaceutical composition of claim 1, wherein said compound is present in an amount of from 0.1%–99% by weight of the total composition.

9. The pharmaceutical composition of claim 1, wherein the carrier is an ointment or a cream.

10. The pharmaceutical composition of claim 1, wherein the compound is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1 -oxyl, the compound is present in an amount of from about 0.01 to about 100 mg/ml of the total composition, and the carrier is an ointment or a cream.

11. The pharmaceutical composition of claim 1, wherein said pharmaceutical composition is a topical composition.

12. A method for treating the effects of oxidative stress due to the production of harmful free radical species, comprising administering a composition comprising an anti-oxidative stress effective amount of a compound selected from the group consisting of the oxidized form of a metal-independent nitroxide, the oxidized form of an oxazolidine compound capable of forming an oxazolidine-1-oxyl, and a physiologically acceptable salt thereof, to an organism or biological material susceptible to oxidative stress.

13. The method of claim 12, wherein the oxidative stress is due to the formation of free radicals by an oxidizing agent, increased oxygen exposure, oxygen therapy, hyperbaric oxygen treatment, oxygen-induced degeneration or disease, reperfusion injury, ionizing radiation, a carcinogenic agent, a chemotherapeutic agent, a mutagenic agent, aging, or arthritis.

14. The method of claim 13, wherein the oxidative stress is due to reperfusion injury.

15. The method of claim 13, wherein the oxidative stress is due to increased oxygen exposure associated with pulmonary adult respiratory distress syndrome.

16. The method of claim 12, wherein the effect of oxidative stress is oxygen-induced lenticular degeneration, cataracts, or hyaline membrane disease in infants.

17. The method of claim 12, wherein the organism is a mammal.

18. The method of claim 12, wherein the compound is administered as a pharmaceutical composition in a form selected from the group consisting of a solution, emulsion, suspension, tablet, capsule, sachet, lozenge, ampoule, ointment, cream, aerosol, powder, granule, eye drops, nose drops spray, suppository, or pessary.

19. The method of claim 18, wherein the pharmaceutical composition is administered parenterally, intramuscularly, subcutaneously, intravenously, intra-articularly, transdermally, orally, bucally, as a suppository or pessary, topically, or as an aerosol spray or drops.

20. The method of claim 19, wherein the composition is administered intravenously at a dose of about 0.1 to about 300 mg/kg/day.

21. The method of claim 19, wherein the pharmaceutical composition is applied topically as a liquid, ointment or cream, and the compound is present in an amount of from about 0.01 to about 100 mg/ml of the pharmaceutical composition.

22. The method of claim 12, wherein the compound is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, and the anti-oxidative stress effective amount is from about 0.1 to about 300 mg/kg/day orally or by intravenous injection, or about 0.1 to about 200 mg/kg/day by inhalation.

23. A method of prolonging the storage life of human or animal cells, tissues, or organs in vitro, comprising contacting said cells, tissues, or organs in vitro with a solution containing an anti-oxidative stress effective amount of a compound selected from the group consisting of the oxidized form of a metal-independent nitroxide, the oxidized form of an oxazolidine compound capable of forming an oxazolidine-1-oxyl, and a physiologically acceptable salt thereof, to protect said cells, tissues, or organs from oxidative stress.

24. The method of claim 23, wherein said compound is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

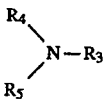
25. A method of inducing weight reduction in humans or animals, comprising administering to a human or animal a composition containing a weight reduction effective amount of a compound selected from the group consisting of a metal-independent nitroxide, an oxazolidine compound capable of forming an oxazolidine-1-oxyl, and a physiologically acceptable salt thereof.

26. The method of claim 25, wherein said compound is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl.

27. A pharmaceutical composition, comprising an anti-oxidative stress effective amount of a compound selected from the group consisting of the oxidized form of a metal-independent nitroxide, the oxidized form of an oxazolidine compound capable of forming an oxazolidine-1-oxyl, and a physiologically acceptable salt thereof, and

a biologically acceptable carrier,

wherein the compound has the formula:



wherein R_3 is selected from the group consisting of –O. and —OH, and R_4 and R_5 combine together with the nitrogen to form a heterocyclic group, or

wherein R_4 and R_5 themselves comprise a substituted or unsubstituted cyclic or heterocyclic group, or a physiologically acceptable salt thereof, said heterocyclic group being selected from the group consisting of a piperidine, pyrrole, imidazole, oxazole,

thiazole, pyrazole, 3-pyrroline, pyrrolidine, pyridine, pyrimidine, purine, and a derivative thereof, and

wherein said pharmaceutical composition is in a form selected from the group consisting of an emulsion, suspension, ointment, cream, aerosol, granule, powder, spray, tablet, capsule, sachet, lozenge, ampoule, pessary, and suppository.

28. The pharmaceutical composition of claim **27**, wherein R_4 and R_5 combine together with the nitrogen to form a substituted or unsubstituted heterocyclic group.

29. The pharmaceutical composition of claim **28**, where R_4 and R_5 combine together with the nitrogen atom to form a substituted or unsubstituted piperidine group.

30. The pharmaceutical composition of claim **29**, wherein R_4 and R_5 combine together with the nitrogen atom to form a piperidine group having one or more methyl substituents and at least one hydroxy substituent.

31. The pharmaceutical composition of claim **30**, wherein said piperidine ring is substituted at the 4-position with a hydroxy group and at the 2- and 6-positions with at least one methyl group.

32. The pharmaceutical composition of claim **27**, wherein the carrier is an ointment or a cream.

33. The pharmaceutical composition of claim **27**, wherein said pharmaceutical composition is a topical composition.

34. The pharmaceutical composition of claim **27**, wherein the compound is 4 -hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, the compound is present in an amount of from about 0.01 to about 100 mg/ml of the total composition, and the carrier is an ointment or a cream.

* * * * *

US006426080B1

(12) **United States Patent**
Golz-Berner et al.

(10) **Patent No.: US 6,426,080 B1**
(45) **Date of Patent: Jul. 30, 2002**

(54) **COSMETIC PREPARATION OF ACTIVE SUBSTANCES WITH HIGH PROTECTION FACTOR AGAINST FREE RADICALS**

(75) Inventors: **Karin Golz-Berner; Leonhard Zastrow**, both of Monaco (MC)

(73) Assignee: **Coty, B.V.**, Haarlem (NL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/720,335**

(22) PCT Filed: **Jun. 22, 1999**

(86) PCT No.: **PCT/DE99/01851**

§ 371 (c)(1),
(2), (4) Date: **Dec. 22, 2000**

(87) PCT Pub. No.: **WO99/66881**

PCT Pub. Date: **Dec. 29, 1999**

(30) **Foreign Application Priority Data**

Jun. 24, 1998 (DE) 198 30 004
Dec. 23, 1998 (DE) 198 60 754

(51) **Int. Cl.**[7] **A61K 6/00**; A61K 31/74; A01N 37/18; A01N 25/00

(52) **U.S. Cl.** **424/401**; 424/78.03; 424/78.05; 514/2; 514/946

(58) **Field of Search** 424/401, 78.03, 424/78.05, 725, 195.17, 195.16, 195.15; 514/2, 788.1, 844–848, 873, 886–87, 904, 905, 937, 944, 945, 946–47

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,402,856 A		9/1983	Schnoring et al.	
5,626,015 A	*	5/1997	Ribier et al.	424/450
5,629,185 A		5/1997	Stanzl et al.	
5,686,405 A	*	11/1997	Lebreton et al.	424/195.15
5,716,599 A		2/1998	Golz et al.	
5,759,969 A	*	6/1998	Tsaur et al.	510/158
5,804,168 A	*	9/1998	Murad	424/59
5,916,577 A		6/1999	Golz et al.	

FOREIGN PATENT DOCUMENTS

DE	34 03 476		8/1984
DE	42 31 544		2/1994
DE	43 28 639		3/1995
DE	42 31 543		6/1995
DE	196 53 736		6/1998
EP	0 507 272		4/1992
EP	0 707 844		4/1995
EP	0 768 079		4/1997
FR	2 597 337	*	1/1987
FR	2 770 228	*	10/1997
JP	58-26809	*	2/1983
JP	8-301722	*	8/1996
JP	09291023		11/1997
WO	93 24106		12/1993
WO	96 06532		3/1996
WO	97/45100		12/1997

OTHER PUBLICATIONS

JPO Abstract, JP408301722A, Aug. 1996.*
Machine translation, JP408301722A, Aug. 1996.*
SU 1780753A1, Kolinko et al., Derwent Abstract, Dec. 1992.*
JP58026809A, Derwent Abstract, Feb. 1983.*
Compton's Encyclopedia Online v3.0, ©1998.*
FR002770228A1, Abstract, Oct. 1997.*
FR 2597337A, Derwent Abstract, Jan. 1987.*

* cited by examiner

Primary Examiner—Michael G. Hartley
Assistant Examiner—Michael A. Willis
(74) *Attorney, Agent, or Firm*—Collard & Roe, P.C.

(57) **ABSTRACT**

The disclosed cosmetic preparation of active substances protects the skin in a particularly effective way against free radical aggression, both alone and in combination with other active substances. The preparation consists of a Quebraco blanco bark extract containing at least 90 wt. % proanthocyanidin oligomers, a silkworm extract containing the peptide cecropin, amino acids and a vitamin mixture, a nonionic, cationic or anionic hydrogel, phospholipids and water, and may also contain further active substances such as vitamin derivatives and plant extracts of acerola, sea weed, citrus, bitter orange, cherry, papaya, tea, coffee beans, Mimosa tenuiflora and angelica. The preparations have protection factors against free radicals of up to 10000, and the cosmetic compositions containing these preparations have protection factors of between 40 and 200, depending on their proportion of the preparations.

12 Claims, No Drawings

COSMETIC PREPARATION OF ACTIVE SUBSTANCES WITH HIGH PROTECTION FACTOR AGAINST FREE RADICALS

The disclosed cosmetic preparation of active substances protects the skin in a particularly effective way against free radical aggression, both alone and in combination with other active substances.

Free radicals such as superoxide ions, hydroxy radicals, oxides are known as a major factor of degeneration and thus the ageing of the skin. They destruct the proteins and lipids of the cellular membrane, affect the DNA and also decompose the hyaluronic acid, a key substance of the skin. Under normal biological conditions there is an equilibrium ratio between the free radicals coming up and their embankment by endogenous chemical or enzymatic systems. Additional outside stress factors such as aggressive atmosphere, tobacco smoke, ultraviolet radiation etc. may overload these inherent immune systems and shift the equilibrium in favour of the free radicals. Inflammation or ageing phenomena of the skin may occur, indicating a need for compensation by cosmetic products.

There has already been proposed a series of products for this purpose, most of them containing mixtures of the vitamins A, C and E or additives of superoxide dismutase or extracts of certain plants or animals. Thus a cosmetic compound containing ultrasound decomposition products of yeast and other cellular dispersions is known from U.S. Pat. No. 5,629,185. From WO96/29048 a cosmetic containing condensed decomposition products of plants or animals is known. There is also a number of publications describing the use of pure plant extracts for cosmetic purposes, such as WO97/45100, where a mixture of seven different extracts is described for anti-cellulite treatment.

The search for other effective substances is a major element of cosmetic research. Another problem of many of these products is that the substances which are effective against free radicals often do not keep their catching properties within the ready cosmetic compound, i.e. it requires special formulations to permanently maintain the effectiveness of the radical catchers.

On the other hand it seems that it has not become widely known in the cosmetic industry yet that there is a possibility of measuring the antioxidant potential of the skin (DE 4328639) and recently also of determining the radical protection coefficient of a cosmetic preparation by using a relatively simple method and to purposefully add materials to such a preparation.

It is an object of the present invention to provide a cosmetic preparation of active substances which has a particularly high radical protection potential.

Another objective of the invention is to provide a preparation of active substances that keeps its radical protection potential over a long period of time.

Another objective of the invention is to provide special cosmetic compounds containing this preparation of active substances and especially such preparations of active substances which achieve further improvement of properties, in particular with regard to opening the pores of the skin.

According to the invention, the cosmetic preparation of active substances with a high radical protection factor is characterised by comprising

(a) a product obtained by extraction of the bark of *Quebracho blanco* and subsequent enzymatic hydrolysis, containing at least 90 percent by weight of proanthocyanidine oligomers and up to 10 percent by weight of gallic acid, wherein the content of (a), which is available in a concentration of 2 percent by weight linked to a microcapsules, ranges from 0.1 to 10 percent by weight;

(b) an extract of the silkworm obtained by extraction, containing the peptide Cecropine, amino acids and a vitamin mix, wherein the content of (b) may range from 0.1 to 10 percent by weight;

(c) a non-ionic, cationic or anionic hydrogel or mixture of hydrogels, wherein the content of (c) may range from 0.1 to 5 percent by weight;

(d) one or several phospholipids comprising 0.1 up to 30 percent by weight;

(e) up to 100 percent by weight of water related to the total weight of the active substance preparation each.

As applicable, the active substance preparation may also contain:

(f) an ultrasound decomposition product of a yeast containing 15 at least 150 units of superoxide dismutase per ml, wherein the content of the decomposition product (f) is in the range from 0 to 4 percent by weight;

(g) an extract of acerola fruits *Malpighia punidifolia*, wherein the content (g) is in the range from 0 to 20 percent by weight; and

(h) a mixture of 0.1 percent by weight of liposomal *Micrococcus luteus* extract, retinyle palmitate and tocopherylacetate prepared with phospholipids and free retinyle palmitate related to the total weight of the active substance preparation each.

For one embodiment of the invention comprising the active substance component (h) the portions of the preparation related to the total weight of the cosmetic are as follows: capsules of the active substance according to (a) ranging from 0.1 to 10 percent by weight, hydrogel according to (b) ranging from 0.1 to 5 percent by weight, encapsulated retinyle palmitate according to (h) 0.001 to 5 percent by weight, encapsulated tocopherylacetate according to (h): 0.001 to 2 percent by weight free retinyle palmitate according to (h): 0.1 to 5 percent by weight, phospholipids: 0.2 to 5 percent by weight, water as the remaining portion up to 100 percent by weight and/or other auxiliary or carrier substances.

The Quebracho bark extract according to the invention or its hydrolysis product has a very high portion of proanthocyanidines representing condensed tannins. These compounds appearing as oligomers and the low portion of gallic acid in this combination and in a concentration between 1 and 10 percent by weight shows a clear radical protection effect, which by far exceeds the effect of superoxide dismutase (SOD). The activity against free radicals was compared with that of SOD and found to be 42% for a 1 percent by weight solution of the extract (SOD 4%), 83% for a 2.5% by weight solution (SOD 15%)and 100% for a 5% by weight solution (SOD 38%). Preferably the extract (a) contains at least 95 percent by weight of proanthocyanidine oligomers and up to 5 percent by weight of gallic acid, in particular at least 99 percent by weight of proanthocyanidine oligomers and up to 1 percent by weight of gallic acid.

The content of (a) is 1 to 10 percent by weight, wherein the active substance from the Quebracho bark is enclosed in microcapsules. The microcapsules may consist of petrolatum, sodium tristearat, agar, phenonip and water.

The silkworm extract (b) is obtained by extraction of the silkworm (*Bombyx mori*) with 1,2-propylene glycol and contains vitamins, amino acids and the Cecropine peptide, which has a special antibacterial functionality. A range of studies of the haemolymph and the cuticular matrix of the

silkworm carried out during the last years showed that it does not only contain antibacterial peptides but also inhibitors, in particular fungal protease inhibitors. Such extracts also show oxygen consuming properties, thus activating the cellular metabolism, and they have moisture-keeping properties, a clear curative effect on lesions in the skin by reducing healing time and a skin-smoothing effect.

Preferably, the extract (b) includes the amino acids aspertinic acid, asparagine, threonine, serine, glutaminic acid, praline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine.

Preferably extract (b) also contains a vitamin mixture including vitamins B_1, B_2, B_5, B_6, B_8, B_9, B_{12}, PP, A, E and C.

The concentration of components (a) and (b) in the active substance preparation preferably ranges from 0.1 to 3 percent by weight each, in particular from 0.5 to 3 percent by weight. The gel contained according to the invention, which may also be a mixture of different gels, is a hydrogel soluble in water at temperatures above 40 up to 50° C., approximately, and which takes the gel structure at low temperatures between 10 and 30° C. Examples of such gels are non-ionic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone-modified maize starch and hydroxyethylcellulose, cationic polymers such as cationic Guar, cationic cellulose, synthetic cationic polymers or gels such as gelatine, carrageenan, bentonite gels, copolymeric gels such as carbomer.

The phospholipids contained according to the invention have been selected among phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, phosphatidic acid and lysolecithines as well as mixtures thereof. Known products are Phoslipone, for example. The contents of phospholipids ranges from 0.1 to 30 percent by weight, preferably 0.5 to 20 percent by weight.

The components (a) and (b) of the active substance preparation and the phospholipids (d) presumably form association-like configurations of different molecules which again are accumulated mostly homogeneously in the generating structure of the gel (c)+(e), the whole being called "association complex". This may also include portions of the SOD-containing yeast decomposition product and of the acerola extract as well as certain plant extracts.

The encapsulated mixture of 0.1 percent by weight of *Micrococcus luteus* extract, retinyle palmitate and tocopherylacetate is present as liposome prepared with phospholipids, wherein the content of retinyle palmitate and tocopherylacetate may preferably range from 0.001 to 1 percent by weight. The portion of phospholipids in this encapsulated mixture generally ranges between 5 and 40 percent by weight.

Additionally, the preparation of the active substance as an association complex may contain a mixture of the vitamins A, B and C as well as additional SOD and/or extracts of acerola fruits. However, the complete preparation of the cosmetic preparation may also contain vitamins and other antioxidants.

According to the invention, the active substance preparation may also contain, in addition to the basic components (a) through (e), different plant extracts such as citrus peel or leaf extracts (*Citrus bigaradia, Citrus hystrix, Citrus aurantifolia, Citrofurtunella microcarpa, Citrus aurantium, Citrus reticulata*), petitgrain extract (peel or fruit), extract of the Spanish cherry, kiwi extract (*Actinidia chinensis*), papaya fruit- extract (*Caricae papayae*), tea extract [leaves of green or black tea, leaves or bark of new jersey tea (*Ceanthus velutinas*)], coffee bean extract (INCI name: coffee bean extract; of green or roasted beans), prunus extract (*Prunus armeniaca, Prunus dulcis, Prunus persica, Prunus domestica, Prunus spinosa, Prunus serotina, Prunus virginiana*), extracts of the bark of the Mexican skin tree (*Mimosa tenuiflora*), angelica root extract (*Angelica archangelica*). Such plant extracts are commercially available, e.g. from DRAGOCO, Holzminden; Germany.

The content of these plant extracts may range from 0 to 40 percent by weight, preferably from 0.1 to 40 percent by weight, in particular 0.5 to 20 percent by weight, where the mixture may also contain mixtures of these extracts as well as mixtures with the components (f) and (g) of the active substance preparation.

Depending on the plant and the added quantity, the addition of the above plant extracts may increase the radical protection factor several times, presumably with the occurrence of synergistic interactions, the correlation between which we have not been able to find out yet completely.

The antioxidants that may be used in the invention include vitamins such as vitamin C and derivatives of it, such as ascorbylacetates, phosphates and palmitates; vitamin A and its derivates; folic acid and its derivates, vitamin E and its derivates, such as tocopherylacetate; flavones or flavonoides; amino acids such as histidine, glycine, tyrosine, tryptophan and derivates of it; carotinoids and carotenes, such as 13-carotin, ct-carotin; uric acid and derivates; a-hydroxy acids such as citric acid, lactic acid, malic acid; stilbenes and their derivates etc.

Vitamins may also be contained in a mixture with enzymes as another portion in the active substance preparation or in the cosmetic composition apart from the active substance preparation. The content may be at least be 0.5 percent by weight of a mixture of enzymes and vitamins containing at least 150 units/ml (U/ml) of superoxide dismutase (SOD).

Preferably, the used mixture of enzymes and vitamins is an ultrasound decomposition product of a yeast, where the decomposition product contains SOD, protease, vitamin B_2, vitamin B_6, vitamin B_{12}, vitamin D_2 and vitamin E. Preferably, it contains at least 150 U/ml of SOD, protease and the vitamins B and D, where the proportion between SOD and protease as international units at least ranges from 3:1 to 8:1.

Of special advantage for making the enzyme/vitamin mixture is an ultrasound-based decomposition method described in DE 4241154C1, where a cellular dispersion or suspension is passed through a continuous ultrasound irradiation cell, where the sonotrode protects up to half or two thirds of its length into the cell and is submerged in the medium to be exposed to ultrasound-irradiation. The sonotrode has an angle of 80.5 to 88.5 degrees, and the correlation between the submerged length in mm of the sonotrode and the exposed volume in ml is set to a value ranging from 1:1.1 o 1:20. The portion of solid particles in the medium to be exposed to acoustic irradiation ranges from 1:0.02 to 1:2.2 percent by weight.

Yeasts such as baker's yeast, brewing yeast, wine yeast as well as specially treated yeasts such as SOD-enriched yeasts can be used as cellular dispersions. For instance, a cellular dispersion that can be preferably used may contain *Saccharomyces cerevisiae.*

The addition, for instance, of 1 percent by weight of such a yeast decomposition product of baker's yeast as an optional portion of the association complex may nearly double a radical protection factor, which itself is high already, from 1620 to 3150. Further remarks on the radical

protection factor will be made below. In addition to the above components the active substance preparation in the form of the association complex may also contain an extract of acerola fruits (*Malpighia punidifolia*). Acerola is a small tree indigenous to the West Indies, to northern South America, to Central America, Florida and Texas, which is rich in vitamin C and other active substances such as Vitamin A, thiamine, riboflavine and niacine, which may develop a complex activity together with different other components such as phosphor, iron, calcium. The aqueous acerola extract is normally available as a powdered product. As another active substance in the complete composition of the cosmetic preparation and in addition to the above active substance complex an especially preferred embodiment may contain one or several of the following components:

(1) extracts or treated extracts of plants binding free radicals or moisture, selected among acerola fruits (*Malpighia punidifolia*), *Camellia oleifera, Colunsonia canadensis and Hibiscus sabdariffa;*

(2) extracts or treated extracts of algae binding free radicals or moisture, selected among omega plankton with a high content of cerebrosid stimulants, micro algae of the chlorella species and macro algae of the ulva species associated with byssus (mussel silk) as biotechnological protein fraction and subsequently associated with dextrine, wherein the product appears in the mixture with peptide derivates derived from a-MSH and associated with xanthin.

(3) natural and synthetic polymers selected among chitosanglycolate, condensed products of desiccated milk, and activated fatty acids,

(4) magnetically hard single crystals of bariumhexaferrite having a coercitive field strength of 3000– 5000 Oe and a grain size of 50–1200 nm intercalated in or mixed with asymmetric lamellar aggregates for phospholipids and fluorocarbons as well as

(5) other active substances and carriers selected among hyaluronic acid, omega CH activator, behentrimonium chloride, passion flower oil as well as modified kaolin.

The mentioned modified kaolin is contained according to W096/17588 and has been modified with spherical TiO_2or SiO_2 particles having a size of <5 μm, wherein the spherical particles's share in the kaolin mixture ranges from 0.5 to 10 percent by weight. This is what makes the preparation feel very smooth on the skin and gives it additional anti-inflammatory functionality. The modified kaoline may amount to a content ranging from 0.1 to 6 percent by weight of the total quantity of the cosmetic.

The mentioned magnetically hard particles for stimulating the circulation of the blood may be such as described in W095/03061 or such with smaller particle sizes and in a mixture with asymmetric lamellar aggregates charged with oxygen up to the saturation pressure, where the content of magnetic particles related to the total composition of the cosmetic may range from 0.01 to 10 percent by weight.

The mentioned asymmetric lamellar aggregates are known from W094/00098 and consist of phospholipids and fluorocarbon charged with oxygen or a fluorocarbon mixture. The fluorocarbon content is in the range from 0.2 to 100 percent by weight/volume, wherein the phospholipid has a phosphatidyl choline content of more than 30 up to 99 percent by weight and where these aggregates have a skin penetration depending on the critical solubility temperature of the fluorocarbons.

In addition, the aggregates may also appear alone in the cosmetic preparation only charged with oxygen. The content may range from 2.5 to 20 percent by weight of the total composition of the cosmetic.

These aggregates are oxygen carriers and allow the penetration of the oxygen into the skin, thus improving oxygen supply to the skin.

The preparation according to the invention further contains cosmetic auxiliary substances and carriers as normally used in such preparations, e.g. water, glycerine, propylene glycol, preserving agents, colorants, pigments with colouring effect, thickeners, softening substances, moisture-preserving substances, aromatic substances, alcohols, polyalcohols, electrolytes, polar and non-polar oils, polymers, copolymers, emulsifiers, waxes, stabilisers, tinted plant extracts such as fat-soluble gardenia extract, fat-soluble carrot extract, paprika LS extract, B-carotene, lithospermum extract and active deodorants.

It is also advantageous to add suitable water-soluble and/or oil-soluble UVA or IVB filters or both to the composition according to the invention. Among advantageous oil-soluble UVB filters are 4-aminobenzoic acid derivates such as the 4-(dimethylamino) benzoic acid (2-ethylhexyl) ester, ester of the cinnamic acid such as the 4-methoxycinnamic acid (2-ethyl-hexyl)ester, benzophenone derivates such as 2-hydroxy-4-methoxybenzophenone, 3-benzylidene camphor derivates such as 3-benzylidene camphor.

Water-soluble UVB filters are for instance sulfonic acid derivates of benzophenone or of 3-benzylidene camphor or salts such as the Na or K salt of the 2-phenylbenzimidazol-5-sulfonic acid.

UVA filters include dibenzoylmethane derivates such as 1-phenyl-4-(4'-isopropylphenyl)propane-1,3-dione.

Preferred solar radiation protection filters are inorganic pigments on the basis of metal oxides such as TiO_2, SiO_2, ZnO, Fe_2O_3, ZrO_2, MnO, Al_2O_3, which can also be used as a mixture with each other or with organic filters. Particularly preferred inorganic pigments are agglomerated substrates of $TiO_2$and/or ZnO, having a contents of spherical and porous SiO_2 particles, wherein the SiO_2 particle size ranges from 0.05 μm to 1.5 μm and where apart from the SiO2 particles there are inorganic particle-type substances of a spherical structure, where the spherical SiO_2 particles form defined agglomerates with other inorganic substances having a particle size ranging from 0.06 μm to 5 μm.

Particularly advantageously used SiO_2 particles are highly monodisperse, non-porous, spherical SiO_2 particles according to DE 3616133, produced by hydrolytic polycondensation of tetraalkoxy silane in an aqueous alcoholic-ammoniacal medium, where a sol of primary particles is generated, which subsequently brings the contained SiO_2 particles to the desired particle size of about 0.05 up to 10 μm by continuously adding tetraalkoxy silane proportioned in a controlled way, depending on the reaction.

Pigments, pigment mixtures or powders with pigment-like functionality, also comprising those having a pearlescent effect, may also comprise substances such as: mica, kaolin, talcum powder, mica-titanium oxide, mica-titanium oxide-iron oxide, bismuth oxychloride, nylon globules, ceramic globules, expanded and non-expanded synthetic polymer powders, powdery natural organic compounds such as pulverized hard algae, encapsulated and non-encapsulated cereal starches and mica-titanium oxide-organic dye.

Normally, a wide range of compounds may be used as softeners, such as stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, 1,2-propanediol, 1,3-butandiol, cetylic alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, oleyl alcohol, isopropyl laurate, decyloleate,

2-octadecanol, isocetylic alcohol, cetylic palmitate, silicon oils such as dimethylpolysiloxane, isopropyl myristate, isopropyl palmitate, polyethylene glycol, lanoline, cacao butter, vegetable oils such as maize oil, cotton seed oil, olive oil, mineral oils, butyl myristate, palmitic acid etc.

Cosmetic preparations with the preparation of the active substance according to the invention may exist as O/W or W/O emulsions. Suitable emulsifiers for O/W emulsions are for instance addition products of 2–30 mol ethylene oxide to linear C_8–C_{22} fatty alcohols, to C_{12}–C_{22} fatty acids and to C_8–C_{15} alkylphenols; C_{12}–C_{22} fatty acid monoesters and diesters of addition products of 1–30 mol ethylene oxide to glycerine Glycerine monoesters and diesters as well as sorbitan monoester and diester of C_6–C_{22} fatty acids, polyol- and polyglycerinester; addition products of ethylene oxide to castor oil; betaines such as coconut alkyl dimethyl ammonium glycinate or coconut acylaminoethylhydroxyethylcarboxymethyl-glycinate (CTFA: cocamidopropyl betaines) as well as ampholytic tensides.

Suitable emulsifiers for W/O emulsions are for instance addition products of 2–15 mol ethylene oxide to castor oil, esters of C_{12}–C_{22} fatty acids and glycerine, polyglycerin, pentaerythrite, sugar alcohols (e.g. sorbite), polyglucosides (e.g. cellulose), polyalkylene glycols, wool alcohols, copolymers of polysiloxan polyalkyl polyether.

The water content of a preparation with the active substance complex may vary within a wide range and is preferably between 5 and 90 percent by weight, where a lower water content of about 0.5–8 percent by weight may be found in particular in lipsticks.

The especially preferable cosmetic preparation with the active substance component (f) is a particularly effective protection against the attack of free radicals on the skin both alone and in combination with other active substances and has a surprising effect on the opening of the pores of the skin, similar to the effect of a cleaning means (peeling). This increases the efficiency of other properties by further ingredients of the cosmetic preparation, like improved moisturizing and smoothing of the skin, thus improving even more and for a longer time the entire state of the skin.

It was also surprising that the retinyle palmitate in both encapsulated and in non-encapsulated form is effective in the upper and lower layers of the skin at the same time and maintains this effectiveness over a longer period of time, thus improving the repair effect of the association complex. The latter seems to be due to the presence of tocopherylacetate, since only in this combination the simultaneous and lasting effect could be observed.

The preparation of the active cosmetic substance according to the invention, when applied either alone or in combination with other active substances, protects the skin in a particularly efficient way against the attack of free radicals on the skin. It has a high radical protection factor between 100 and 3500×10^{14} Radicals/mg.

The radical protection factor (RPF) determines the activity of a substance for binding free radicals as compared with a test substance. The test substance consists of a highly reactive, semi-stable radical, which reacts with all known antioxidants. Such radicals include nitroxides such as proxo (2,2,5,5-tetramethyle-1-dihydropyrrolinoxy-nitroxide), tempol (2,2,6,6-tetramethyl-1-piperidinoxy-4-ol-nitroxide), DTBN (ditert-butyl-nitroxide) or preferably DPPH (1,1-diphenyl-2-picryl-hydrazyl).

The RPF is determined by measuring the signal amplitude of the test radical by electron spin resonance (ESR/EPR) before and after mixing with an antioxidant and by calculating the RPF on this basis. For a series of standard antioxidants the RPF is a known parameter, so it is 827 for all-trans-retinole, 196 for all-trans retinal acetate, 41200 for DL-α-tocopherol and about 48 for a-tocopherol acetate, each $\times10^{14}$ radicals/mg.

The preparation of the active cosmetic substance alone, if existing as "association complex" of the components (a) through (e) and in a concentration of 10 percent by weight of (a) and (b) each has an RPF of 1255, which is very high as compared with common active substances in cosmetic formulations with declared radical scavengers, which achieve values of about 4 to 40. This is the case even though the concentration of the active substances themselves in (a) and (b) is only 2 percent by weight, as a maximum. "High radical protection factor" according to the present invention means a value of 100 or higher, preferably 1000 or higher. In certain combinations of plant extracts and the association complex itself according to the present invention this value may be increased to 10000 and higher. Depending on the portion of the preparation, the corresponding cosmetic compositions with such preparations comprise radical protection factors, for example, from 40 to 200 or higher. The exact method for measuring the radical protection factor has been described by Herrling, Groth, Fuchs and Zastrow in Conference Materials "Modern Challenges To The Cosmetic Formulation" 5.5.-7-5.97, Düsseldorf, p. 150–155, Verlag f. chem. Ind. 1997. Starting from the known concentration of the test substance (here: DPPH) or the number of its free radicals (radicals per ml) they measure a signal amplitude S_1 with an ESR spectrometer. The test radical and the antioxidant are dissolved in a water/alcohol solution (e.g. 0.1 m) each. The signal amplitude S_2 of the antioxidant is measured. The normalised difference between the two signal amplitudes is the reduction factor RF.

$$RF=(S_1-S_2)/S_1$$

The result of the radical reduction of the test substance RC ×RF is normalised relative to the quantity of product input PI (mg/ml). Where RC is the amount of the test substance, i.e. the known number of radicals in the test substance. The radical protection factor is calculated by means of the following equation:

$$RPF=\frac{RC\ [\text{Radicals/ml}]\times RF}{PI\ [\text{mg/ml}]}$$

The result is

$$RPF=N\times10^{14}[\text{Radicals per mg}],$$

where N is a positive real number and RPF for simplification may be reduced to the value of N. This reduction has been used in the examples of the present invention.

The radical protection factor may be determined by means of a handy and very simple ESR spectrometer (GALENUS GmbH, Berlin, Germany) and is a new magnitude for characterising cosmetic products as regards their capacity of binding free radicals. The method is an in vitro method, where no individual properties of the user of the cosmetic are influencing the antioxidants.

Other advantageous effects of products with the active substance preparation according to the invention, in combination with other active substances or carriers are a lasting improvement of the general state of the skin, a delayed ageing process of the skin, lasting improvement of the moisturizing and smoothing effect on the skin. The particularly advantageous embodiment described above with an

additional algae-peptide product and magnetically hard single crystals of bariumhexaferrite comprises a special allergy-reduced risk, according to allergy and dermatological tests.

The cosmetic preparation according to the invention may be used, for example, in sun creams, sun gels, after-sun products, day creams, night creams, masks, body lotions, cleansing milk, makeup's, lipsticks, eye cosmetics, hair masks, hair conditioners, shampoos, shower gels, shower oils, bathing oils and other common products. A particular advantage of the active substance preparation according to the invention is the embodiment with the optional component (f) in a cream, lotion, a makeup, fluid, gel or lipstick. Advantageous cosmetic preparations also include tooth pastes mouthwash, under the special aspect of neutralising free radicals in the mouth of smokers and also as special cream for the hands and the face of smokers. Such products are manufactured in a way known by workers skilled in the art. When selecting special carrier substances, the corresponding pharmaceutical preparations may also be made.

Another subject matter of the invention is a cosmetic preparation comprising a content of plant extract selected from the group comprising citrus extract, petitgrain extract, cherry extract of the Spanish cherry, papaya fruit extract, tea extract, coffee bean extract, prunus extract, skin tree extract, angelica extract and mixtures of them as has been defined more in detail above, with a content of 0.5 to 40 percent by weight as well as 99.5 to 60 percent by weight of other active substances or carriers or mixtures of active substances and carrier substances, each related to the total composition. Active and carrier substances may be the substances mentioned above.

The following examples are to illustrate the invention more in detail. If not otherwise indicated, all measures will be given in percent by weight.

Manufacture of the Active Substance Complex

For making the gel basis, gel powder such as carbomer was added to water, homogenised and subsequently neutralised with triethanolamine, for example. Then ethanol and glycerine were added to improve mixing properties, and the mixture was well stirred.

To this gel basis a mixture of phospholipids (Phoslipone), Quebracho extract and silkworm extract was added and mixed at a temperature of up to 45° C. Then another portion of the above gel or a second gel such as Guar propyl triammonium chloride was added and stirred well with the whole mixture at increased temperature, but below 45° C. This way you got the active substance preparation according to the invention, hereinafter called "complex".

In those cases where the active substance preparation contained other ingredients such as yeast decomposition product, acerola extract or extracts of tea, coffee, kiwi, citrus, cherry, papaya or skin tree, such extract was added to the mixture of phospholipids and mixed with the gel.

EXAMPLE 1

Day cream

phase A: carbomer 0.2; glycerine 2.0; propylene glycol 1,0; dist. water q.s. ad 100;

phase B: C_{12}–C_{15}-alkyl cetylic alcohol 3,7; stearate 0,5; jojoba oil 1,0;

phase C: triethanolamine 0,2;

phase D: active substance complex with (a) through (f) 3.5perfume 0.5; preservant 0.3

Phases A and B were warmed up to 65±2° C. while being stirred, and phase B was homogenised in phase A. Then phase C was added and homogenised correspondingly. Subsequently, the mixture was cooled down to 35° C. while being stirred, and phase D was added and mixed thoroughly. The active substance complex contained 1.0% of an SOD-containing enzyme/vitamin product obtained from baker's yeast using the ultrasound method according to DE 4241154C1.

The added active substance complex contained 1% of dry gel, 7% of phospholipids, 2% of Quebracho extract, 1% of silkworm extract, 1% of SOD from a yeast decomposition product. The radical protection factor of this active substance complex amounted to 1925, and in the formulation the RPF was around 49.

EXAMPLE 2

Special Cream

phase A: glycerine 3.0; dist. water q.s. ad 100;

phase B: Vaseline 22.5; jojoba oil 5.0;

phase C: active substance complex with (a) through (f) 5.5; asymmetric lamellar aggregates (AOCS) according to example 2 of W094/00109 10.5; AOCS with Ba hexaferrite single crystals according to example 1 of W095/03061, 2.0; modified kaolin according to example 1 of W096/17588 0,3.

Phases A and B were warmed up to 65±2° C. while being stirred, and phase B was homogenised in phase A. Subsequently, the mixture was cooled down to 35° C. while being stirred, and phase C was added and mixed thoroughly.

The active substance complex contained 1.0 % of an SOD-containing enzyme/vitamin product obtained from wine yeast using the ultrasound method according to DE 4241154C1 and in addition 0.5% of vitamin E and 0.5% of vitamin C. The basic ingredients of the active substance complex were 0.5% of dry gel, 10% of phospholipids, 1% of Quebracho extract, 2% of silkworm extract. The radical protection factor of this active substance complex amounted to 2120, and in the formulation the RPF was around 41.5.

EXAMPLE 3

Sun Gel

phase A: carbomer 1.5; glycerine 3.0; propylene glycol 2.5; dist. water q.s. ad 100;

phase B: triethanolamine 1,5;

phase C: peptide preparation MAP-XE according to PCT/DE97/02941 1,0; ZnO 2.0; TiO_2 5.0; SiO_2 1.0; shellac (20% aqueous solution) 1.0;

phase D: active substance complex with (a) through (f) 3,5;

phase E: perfume 0.5, preservant 0,3.

Phase A was warmed up to 60±2° C. while being stirred and homogenised and cooled down to 45° C. Phase B was homogenised in phase A. After cooling down to about 40° C., phase C was added and the mixture was well homogenised. Subsequently, the mixture was cooled down to 35° C. while being stirred, and phases D and E were added and mixed and stirred thoroughly. The active substance complex contained 1.0 % of an SOD-containing enzyme/vitamin product obtained from brewer's yeast using the ultrasound method according to DE 4241154C1 and in addition 0.5 % of vitamins E, vitamin C and vitamin A, each. The basic ingredients of the active substance complex were 0.15% of dry gel, 20% of phospholipids, 5% of Quebracho extract, 3% of silkworm extract. The radical protection factor of this active substance complex amounted to 3050.

EXAMPLE 4

Day Cream

A composition according to example 1 was made, with the active substance complex containing 20% of black tea extract instead of 1% of SOD of a yeast decomposition. The radical protection factor of this active substance complex amounted to 3100.

EXAMPLE 5

Sun Cream

A composition according to example 1 was made, wherein phase A contained an additional 3% of TiO_2 and 7.5% of benzophenone-3. Instead of 1% of SOD of a yeast decomposition, the active substance complex contained 5% of coffee bean extract of roasted coffee beans and 2% of kiwi extract. The radical protection factor of this active substance complex amounted to 3200.

EXAMPLE 6

Day Cream

A composition according to example 1 was made, wherein phase A contained an additional 1% of TiO_2 and 0.5% of ZnO. Instead of 1% of SOD of a yeast decomposition, the active substance complex contained 1% of green tea extract, 2% of an extract of green coffee beans, 1% of vitamin C and 1% of vitamin E (tocopherolacetate). The radical protection factor of this active substance complex amounted to 5600.

EXAMPLE 7

Example for Comparison Purposes

The following components of an active substance complex were mixed with each other:

0.15% of guar propyl triammonium chloride (gel); 20% of phospholipids; 0.1% of triethanolamine; 1.0% of vitamin E; 0.1% of vitamin C; 78.65% of water.

The measured radical protection factor of the whole compound was 2.

EXAMPLE 8

Emulsion-based Fluid with Increased Vitamin A Content (Vitamin A^2)

phase A: carbomer 0.05, glycerine 2.5, propylene glycol 0,5; dist. water q.s. ad 100;

phase B: C_{12}–C_{15}-alkyl cetylic alcohol 1.5; stearate 0,1; olive oil 1,0;

phase C: triethanolamine 0,05;

phase D: complex with (a) through (d) containing 2% of quebracho extract, 2% of silkworm extract, 0.1% of carbomer, 0.1% of TEA, water 0.9%; as well as (h) retinyle palmitate and tocopherylacetate (1:1) as liposomes with phospholipids with 0.1% of *Micrococcus luteus* extract =0.1, retinyle palmitate (free) 0.5; phase E: perfume oil 0.2, preservant 0.3.

Phases A and B were warmed up to 65±2° C. while being stirred, and phase B was homogenised in phase A. Then phase C was added at about 50° C. and homogenised correspondingly. Subsequently, the mixture was cooled down to about 30° C. while being stirred, and phases D and E were added and mixed and homogenised thoroughly.

The radical protection factor of the complex amounted to 2100, and in the fluid the RPF was around 20.

EXAMPLE 9

O/W Antismoke Day Cream

phase A: sorbitan monostearate 4; avocado oil 3; Oleyl oleate 8;

phase B: water q.s. ad 100; propylene glycol 2, glycerine 5 carbomer 0.2;

phase B1: NaOH 0.4;

phase C: preservant 0.4;

phase D: active substance complex with (a) through (f) with vitamins A,E,C,B 5;

phase E: perfume oil 0,5.

Phases A and B were made separately at 80° C. while stirring intensively and subsequently mixed, stirred and homogenised. After cooling down to 60° C., phase B1 was added for neutralisation. After cooling down to 50° C., phase C was added. At 30° C. phases D and E were added to the mixture one after another and homogenised; RPF=39.

EXAMPLE 10

Antismoke Night Cream

The cream provides a repair effect to a smoker's skin while at the same time having a prophylactic effect for the day.

phase A: Vaseline 8.5; jojoba oil 3.0; stearic acid 3.8;

phase B: water q.s. ad 100; glycerine 5; carbomer 0.3;

phase C: triethanolamine 0,3;

phase D: preservant 0.4;

phase E: active substance complex (a) through (f) with vitamins A, E, C, B and 2% aloe vera 10.0; perfume oil 0.1.

The procedure was the same as in example 9; RPF =48.

EXAMPLE 11

Hand Cream Against Brown Smoker's Fingers

phase A: cetylic alcohol 8.5; stearic acid 3.8;

phase B: water q.s. ad 100; glycerine 2, carbomer 0.9;

phase C: triethanolamine 0,3;

phase D: vitamin E 1.0; aloe vera 1.0; preservant 0.4; active substance complex with (a) through (f) with vitamins A, E, C 5.0; perfume oil 1.4; whitening complex 1,0.

The procedure was the same as in example 9; RPF=35.

What is claimed is:

1. Cosmetic active substance preparation with a radical protection factor, which comprises a content of

(a) a product obtained by extraction of the bark of *Quebracho blanco* and subsequent enzymatic hydrolysis, containing at least 90 percent by weight of proanthocyanidine oligomers and up to 10 percent by weight of gallic acid, where the content of (a), in the cosmetic active substance prepartion ranges from 0.1 to 10 wt. % and wherein (a) is present as a microcapsule with a concentration of the extraction product of 2 wt %;

(b) an extract of silkworm obtained by extraction, containing the peptide cecropine, amino acids and a vitamin mix, where the content of (b) ranges from 0.1 to 10 wt. %;

(c) a non-ionic, cationic or anionic hydrogel or mixture of hydrogels, where the content of (c) ranges from 0.1 to 5 wt. %;

(d) at least one phospholipid in the range of 0.1 up to 30 wt. %;

(e) water,

wherein the radical protection factor is in the range 100 to $3500 \cdot 10^{14}$ radicals per mg preparation; and wherein an association complex is between the phospholipids (d), at least comprising the components (a) and (b) and the gel (c) with the water (e).

2. Preparation according to 1 further comprising

(f) an ultrasound product of a yeast containing at least 150 International units of superoxide dismutase per ml, wherein the content of the decomposition product (f) is in the range from 0.1 to 4 wt. %, and

(g) an extract of acerola fruits *Malpighia punidifolia*, wherein the content (g) is in the range from 0.1 to 30 wt. %; related to the total weight of the active substance preparation each.

3. Preparation according to 2 further comprising

(h) a mixture of 0.1 wt. % if *Micrococcus luteus* extract, retinyl palmitate and tocopherylacetate in liposomal

form prepared with phospholipids, and additionally tree retinyl palmitate and where the portion of the components contained in the preparation relative to the total weight of a cosmetic preparation are as follows: product of (a) and extract of (b) ranging from 0.1 to 10 wt. %, hydro gel according to (c) ranging from 0.1 to 5 wt. %, encapsulated retinyl palmitate according to (h) ranging from 0.001 to 5 wt. % encapsulated tocopherylacetate according to (h) 0.001 to 5 wt. %, tree retinyl palmitate according to (h) 0.1 to 5 wt. %, phospholipids 0.2 to 5 wt. %.

4. Preparation according to claim 3,

wherein the portions of the components lie within the following ranges: product of (a) and extract of (b) ranging from 0.5 to 3 wt. %, hydro gel according to (c) ranging from 0.1 to 3 wt. %, encapsulated retinyl palmitate according to (h) ranging from 0.05 to 2 wt. %, encapsulated tocopherylacetate according to (h) ranging from 0.05 to 1 wt. %, free retinyl palmitate according to (h) ranging from 0.5 to 2 wt. %.

5. Preparation according to claim 1,

wherein a radical protection factor in the range from 1000 to $3500 \cdot 10^{14}$ radicals per mg measured by determining the number of free radicals of a solution of a test substance (S_1) by electron spin resonance (ESR) as compared with the ESR measurement result of the cosmetic active substance preparation according to the relationship RPF=(RC×RF)/PI, where $RF=(S_1-S_2)/S_1$; RC=concentration of the test substance (radicals per ml); PI=concentration of the active substance preparation (mg per ml).

6. Preparation according to claim 1,

wherein the product (a) contains at least 99 wt. % of proanthocyanidine oligomers and up to 1 wt. % of gallic acid.

7. Preparation according to claim 1,

wherein the amino acids contained in (b) are selected from the group consisting of aspertine acid, asparagine, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine.

8. Preparation according to claim 1,

wherein the vitamin mixture included in (b) comprises the vitamins B_1, B_2, B_5, B_6, B_8, B_9, B_{12}, PP, A, E and C.

9. Preparation according to claim 1,

wherein the active substance preparation contains an additional mixture of the vitamins A, E and C or each of these vitamins individually.

10. Preparation according to claim 1,

wherein the active substance preparation is a cosmetic composition further comprising at least one of the following components:

(1) extracts binding free radicals or moisture of

(1.1) plants selected from the group consisting of acerola fruits (*Malpighia punidifolia*), *Camellia Oleifera, Colunsonia canadensis* and *Hibiscus sabdariffa*; or

(1.2) algae selected from the group consisting of omega plankton, providing a high portion of cerebrosid stimulants, microalgae of the chlorella species and macro algae of the ulva species with byssus (mussel silk) as biotechnological protein fraction and subsequently associated with dextrine, wherein the product is in the mixture with peptide derivates derived from a-MSH and associated with xanthin;

(2) yeast decomposition products selected from the group consisting of baker's yeast, brewer's yeast, wine yeast and made according to an ultrasound treatment of the aqueous yeasts;

(3) natural and synthetic polymers selected from the group consisting of chitosanglycolate, condensed products of desiccated milk, and activated fatty acids;

(4) magnetically hard single crystals of bariumhexaferrite having a coercitive field intensity of 3000–5000 Oe and a grain size of 50–1200 nm intercalated in or mixed with asymmetric lamellar aggregates of phospholipids and fluorocarbons; and

(5) other active substances selected from the group consisting of chitosanglycolate, hyaluronic acid, omega CH activator, behentrimonium chloride, passion flower oil and carrier substances;

(6) mixtures thereof.

11. Preparation according to claim 1,

wherein the concentration of the product (a) and extract (b) in the active substance ranges from 0.1 to 3 wt. % each.

12. Preparation according to claim 1,

wherein the preparation comprises an additional portion if 0.1 to 20 wt. % of plant extracts selected from the group consisting of citrus peel or leaf extracts (*Citrus bigaradia, Citrus hystrix, Citrus aurantifolia, Citrofurtunella microcarpa, Citrus aurantium, Citrus reticulata*), petitgrain extract (peel or fruit), extract of the Spanish cherry, kiwi extract (*Actinidia chinensis*), papaya fruit extract, (*Caricae papayae*), tea extract [leaves of green or black tea, leaves or bark of tea (*Ceanthus velutinas*)], prunus extract (*Prunus armeniaca, Prunus dulcis, Prunus persica, Prunus domestica, Prunus spinosa, Prunus serotina, Prunus virginiana*), extracts of the bark of the Mexican skin tree (*Mimosa tenuiflora*), angelica root extract (*Angelica archangelica*); and the remaining portion of carrier substances.

* * * * *